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MULTIPLE MYELOMA

A Survey Based on Thirty-Five Cases, Eighteen of Which Came to Autopsy

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MULTIPLE MYELOMA (myelomatosis) is a clinically and anatomically distinctive malignant disease of the skeleton which apparently takes its departure from the myeloid formative tissue proper. The disease is by no means rare. It seems probable that many cases go unrecognized. The condition is more common in males than in females, but not strikingly so. It shows a predilection for persons who are between 40 and 60 years of age, and though it is not unusual in those who are in the 30's, it is definitely uncommon in persons who are less than 30 years of age.

Anatomically, practically every bone may ultimately come to be involved, more or less, in a given case. The progress of the disease may be steady and rapid, sometimes from the beginning and sometimes after a static period. One gathers also that in some cases, before the disease becomes spread over the skeleton, it may flourish in one bone (as a so-called solitary myeloma) for months or even years. Though at autopsy the skeleton may be found riddled through with foci of myeloma, it is only infrequently that gross myelomatous foci are found in the viscera and other extraskeletal parts. Nevertheless, even in the absence of gross infiltrations, microscopic examination sometimes reveals the presence of smaller or larger numbers of myeloma cells within the spleen, the liver or the lymph nodes, and occasionally in other organs as well. Also, in some cases myeloma cells invade the blood stream. Ordinarily, under these circumstances, relatively few myeloma cells are found in the blood smears. However, in an occasional case they may be so numerous as to create a leukemic blood picture (so-called plasma cell leukemia).

This discussion of multiple myeloma is based on 35 cases, in all but 3 of which the diagnosis of myeloma was confirmed by examination of tissue. In the 3 excepted cases, although Bence Jones proteinemia was found in only 1, the diagnosis seemed fairly certain from the fact that

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hyperglobulinemia or hypercalcemia or both were in association with roentgenographic skeletal changes consistent with multiple myeloma. In the great majority of our cases the roentgenographic examination was not limited to the skeletal region giving clinical difficulties but, indeed, covered a substantial part of the skeleton, so that the full extent and distribution of the tumor process was mirrored. In more than half the cases, particularly in those observed in recent years, chemical estimations of serum albumin and globulin and of serum calcium were made. In all but a few of the cases the urine was examined for Bence Jones protein, often repeatedly. Eighteen of the 35 patients came to autopsy. The examination of these 18 included a detailed study of the skeleton. Specifically, much of the vertebral column, the sternum, many of the ribs, parts or all of the pelvic bones and, in many instances, some of the long bones and the calvarium were removed and cut open. Furthermore, in many instances the sectioned bones were then roentgenographed, and in all instances tissue was removed from many representative areas and studied microscopically.

CLINICAL CONSIDERATIONS

Age and Sex Incidence.—Fully three fourths of the patients were between 40 and 60 years of age. This finding is in accord with the generally accepted view that multiple myeloma shows a predilection for persons who are in later middle life. The incidence of the disease appears to fall off sharply in persons who are past 60. As to its incidence in persons less than 40 years of age, as noted, it is by no means unusual in those who are in the 30's but is rather rare in those who are less than 30. Only 1 of our patients was below this age, the one in question being a boy of 17, who presented the first manifestations of the disease at the age of 13. A few unequivocal instances of multiple myeloma occurring in adolescents or children have also been reported.[1] Some others, less convincing, in the literature may actually be instances of Schüller-Christian disease, stem cell leukemia, Ewing's sarcoma or neuroblastoma that has metastasized to the skeleton.

As noted, our data indicate that multiple myeloma may be slightly more prevalent in males than in females but do not support the often repeated statement that the condition is at least twice as frequent in males.

Clinical Complaints.—Evaluation of the clinical records of our cases showed that the major difficulties (occurring singly or in combination) which brought the patients to the hospital were, in the order of decreasing frequency: (1) pain, especially of the back and the thorax, (2) substan-

1. Gordon, H., and Schneider, B.: Internat. Clin. **4**:173, 1940. Berkheiser, E. J.: Arch. Surg. **8**:853, 1924. Laurentius, P.: Monatschr. f. Kinderh. **73**:95, 1938.

tial loss of weight, (3) pathologic fracture of some bone and (4) a palpable tumor appearing in relation to a superficial flat bone. It should be pointed out, however, that patients seeking admission to our hospital tend to do so mainly because of complaints directly referable to skeletal alterations rather than because of anemia, abnormal bleeding, neurologic symptoms or findings suggesting nephritis.

The pain occurring in the back and the thorax was often vague and generalized, and accompanied by a feeling of weakness. However, in the presence of compressed or collapsed vertebral bodies it had a more localized, persistent and disabling character and was frequently associated with some manifestations of compression of the spinal cord or at least of issuing nerve roots. The loss of weight amounted to 30 pounds (13.5 Kg.) or even more in some cases. As the disease progressed, there was a tendency, even in those who showed no appreciable loss of weight on admission, toward increasing debilitation and terminal emaciation. Pathologic fracture as expressed in compression collapse of one or more vertebral bodies was not uncommon, but in some cases a femur or a humerus, for instance, presented a pathologic fracture extending through an area of exuberant myelomatous involvement. If one or more tumor masses are palpable on the skeleton, they are most likely to be found in relation to a clavicle or to one or several ribs. However, a tumor may sometimes be palpable in relation to such superficial bone sites as the calvarium or the facial bones, the sternum, a scapula or an innominate bone.

Presenting Skeletal Lesions.—There are some cases of multiple myeloma in which the patients complain of rather generalized bone pain without specific localization, in spite of widespread skeletal involvement. Usually, however, it is difficulty with some particular bone or skeletal region that first directs attention to the presence of the disease. The involved site most often responsible for difficulties in those whose initial skeletal complaints were localized was the vertebral column. Indeed, in approximately one half of these patients the difficulties were referable to the spine. The lumbar region was most often affected, next the dorsal and least often the cervical region. Patients having complaints referable to the vertebral column often showed roentgenographic evidence of compression or collapse of one or more vertebral bodies on admission to the hospital, and a number of them presented signs of resulting compression of the cord as well (fig. 3 *C*). It seems worth mentioning that, with involvement of the lumbar region of the spine, disability and sciatic pain were common complaints even in the absence of compressed or collapsed vertebral bodies.

The long bones, particularly the femur, represented another clinically important site of localization. Specifically, there were 5 patients each of whom presented in a femur a strikingly large focus of myeloma that

rendered the area easily susceptible to pathologic fracture. This large focus of destruction was found sometimes in the midshaft and sometimes at the end of the shaft. Roentgenographically, the femoral lesions were regularly misinterpreted as representing something other than multiple myeloma until their true nature was established by biopsy (fig. 8 *D*). Large, destructive lesions comparable to those observed in femurs were also observed occasionally in humeri.

When it occurs in a superficially located bone, the presenting lesion is also likely to be clinically palpable. Thus, not infrequently, such a

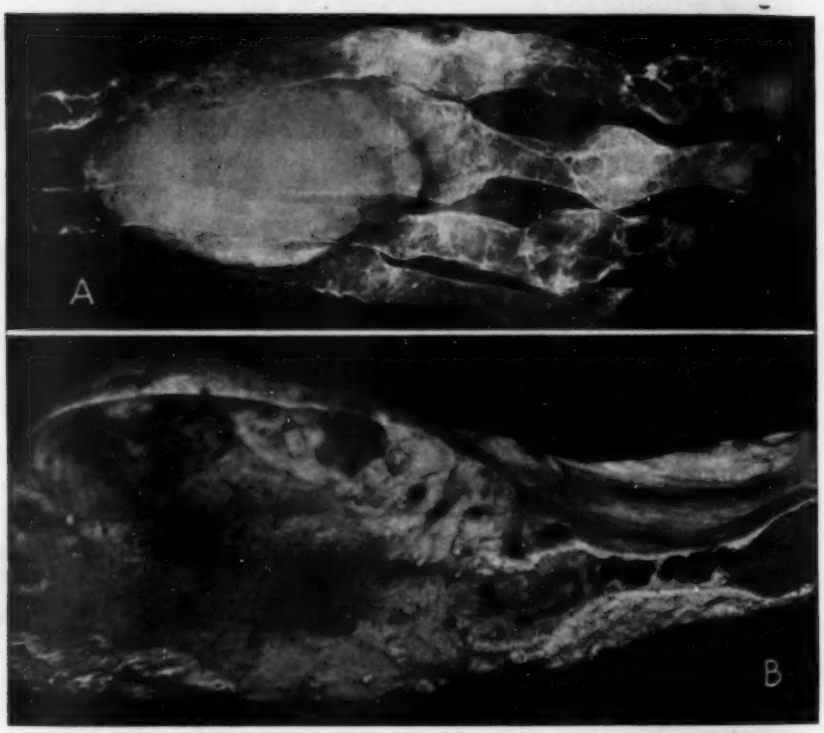

Fig. 1.—*A*, roentgenogram (reduced) of myeloma involving several ribs in a case of far advanced multiple myeloma in which an autopsy was made. A large parosteal tumor mass is seen where the myeloma has extended through the cortex of one of the ribs. The ribs are extremely porotic, expanded, and honeycombed by tumor tissue. This subject was known to have had multiple myeloma for at least nine years.

B, photograph of the expanded tumor shown in *A*. The tumor tissue is hemorrhagically mottled in places. The pleura serves as an effective restraining barrier, but on the external surface the neoplastic tissue has extended beyond the periosteum into the adjacent muscle and connective tissue.

tumor can be felt on a rib or a clavicle (fig. 1). Actually, however, even a bone of a foot or a hand may sometimes be the site of the presenting lesion.

Clinical Course and Prognosis.—In many cases the disease pursues an insidious course in the beginning, the patient complaining merely of some weakness, loss of weight or vague pain in the back or the chest. Sometimes, a fracture of some bone (commonly a rib or a limb bone) weakened by an exuberant focus of tumor within it is the first major difficulty. In other instances the disease may be ushered in dramatically by sudden onset of severe root pain or even paraplegia resulting from compression or collapse of one or more vertebral bodies. Taking our cases altogether, we find that the duration of symptoms prior to hospitalization ranged from as short a time as a few weeks to as long as two years, the average duration being about nine months.

Whatever the initial complaints that bring the patients to the hospital, the progress of skeletal involvement thereafter, as judged by successive roentgenograms, is rather variable and frequently unpredictable. The patients who present a far advanced stage of the disease when first observed usually (though not invariably) survive no more than some weeks or months. As for those with still limited involvement roentgenographically, in some one observes tremendous progression within just a few months, while from others one gains the impression that the neoplasm progresses relatively slowly or remains static for months or even a number of years before entering its terminal phase. This variability of tempo is not explainable on a cytologic basis alone. The average period of survival following the onset of symptoms was about two years in our cases, which is in accord with the general impression, but there are striking deviations from this average. Two patients in whom transverse myelitis developed succumbed within one and three months, respectively, although it is true that neither of them received the possible benefit of laminectomy for decompression. On the other hand, we did an autopsy on a patient whose history of the disease dated back over nine years, and another patient (presenting tumor in an ilium initially) is still alive and comparatively well after ten years. While these instances are exceptional, it is well known that the clinical course of multiple myeloma may occasionally be protracted and characterized by long remissions, which are frequently attributed to roentgen treatment but which may be spontaneous. Cases in point have been reported by Gross and Vaughn,[2] Kirsch,[3] Batts[4] and Davison and Balser.[5] The latter cited an extraordinary case which came to autopsy sixteen years after the onset of symptoms. It is true also that patients in whom the tumor is apparently localized in some one bone in the beginning not infre-

2. Gross, R. E., and Vaughn, W. W.: Am. J. Roentgenol. **39**:344, 1938.
3. Kirsch, I. E.: M. Bull. Vet. Admin. **18**:96, 1941.
4. Batts, M., Jr.: Arch. Surg. **39**:807, 1939.
5. Davison, C., and Balser, B. H.: Arch. Surg. **35**:913 and 935, 1937.

quently go on for a number of years before the myeloma becomes disseminated over the skeleton.[6]

In most cases, as the disease progresses there is a tendency toward demineralization and devastation of the skeleton, associated with increasing anemia and cachexia, provided the clinical course is not cut short, as it frequently is, by such complications as intercurrent infection (especially pneumonia in bedridden patients), concomitant cancer of some other kind, cardiac failure, renal insufficiency, ascending infection of the urinary tract and amyloidosis.

PROBLEMS OF DIAGNOSIS

There are numerous cues to the diagnosis of multiple myeloma coming from many quarters—clinical, roentgenographic, hematologic, biochemical —and the recognition of the condition prior to biopsy or postmortem examination frequently requires their fullest utilization. It is true that the diagnosis of multiple myeloma will be fairly obvious if many bones, including the calvarium, are veritably riddled by osteolytic defects (the picture usually stressed in the texts) and if, in addition, Bence Jones proteinuria is discovered. Unfortunately, this skeletal picture represents the exception rather than the rule, in our experience at least, and Bence Jones proteinuria is as likely to be absent as present. Indeed, often one observes merely some vaguely defined rarefactions in some of the bones or a single exuberant tumor in a single bone without obvious involvement of the skeleton generally, and sometimes (when myelomatous infiltration of the bone marrow is diffuse) skeletal changes may not be apparent at all roentgenographically in spite of complaints referable to the skeleton. In such equivocal or initially obscure cases the lead may come from the discovery of anemia, the presence of myeloma cells in blood smears, hypercalcemia, hyperproteinemia (and certain peculiar hematologic manifestations resulting from increase of serum globulins), evidences of renal damage of a peculiar type or even from the finding of unusual tumor-like amyloid deposits. It must be recognized, however, that these pertinent findings are not all present in every case or necessarily present in the early stages of the evolution of any given case, nor are they necessarily pathognomonic in themselves. It is only by utilizing all the logical approaches that one is likely to arrive at a combination of significant findings constituting probable or conclusive evidence of the presence of multiple myeloma.

For anatomic confirmation, puncture of the sternal marrow should be freely employed. A high degree of reliability is claimed for this procedure, though there are undoubtedly cases of multiple myeloma in

6. (*a*) Bailey, C. O.: Am. J. Roentgenol. **36**:980, 1936. (*b*) Pasternack, J. G., and Waugh, R. L.: Ann. Surg. **110**:427, 1939. (*c*) Stewart, M. J., and Taylor, A. L.: J. Path. & Bact. **35**:541, 1932.

which sternal marrow spreads fail to yield significant information. Biopsy of some obviously affected and readily accessible bone will resolve any possible doubt as to the diagnosis, since the histologic recognition of a myeloma entails no difficult problems in differential diagnosis as a rule.

SKELETAL ALTERATIONS AND THEIR ROENTGENOLOGIC INTERPRETATION

No single description can do justice to the gross appearance of the bones in all cases of multiple myeloma coming to autopsy. At one extreme there is the occasional case in which the bones appear normal as to surface and contour and those removed do not even offer any striking lack of resistance when being cut open. On inspection of the cut surfaces of these bones, one finds that the spongy trabeculae are still numerous and that the cortices are not significantly thinned. However, the marrow is modified and replaced more or less diffusely by rather whitish tissue which on histologic examination is proved to be myeloma tissue. In conformity with these findings, the skeletal roentgenograms taken during life in such a case may show at most some diffuse porosity of the bones. They certainly show nothing even remotely suggestive of the roentgenographic picture one has been taught to regard as representative of multiple myeloma. This was the skeletal status in a case of atypical amyloidosis and myeloma which we studied. It reemphasizes the fact that in every case of atypical amyloidosis, despite the lack of evidence of multiple myeloma in the clinical roentgenograms, bones must be opened and their marrow examined histologically to establish or rule out the presence of myeloma.

In another occasional case, while the bones show smooth and undistended contours, they cut with abnormal ease. When cut, such bones show thinning of the cortices from the medullary side, as well as great reduction of the spongy trabeculae. This is the result of encroachment on the osseous tissue by a gray-whitish tissue which has substantially replaced the marrow and which stands out in some places as discrete foci of tumor. The clinical roentgenograms of the bones in such a case hardly suggest the conventional idea of the roentgenographic picture of multiple myeloma but do reflect the tumor encroachment on the osseous tissue by showing vague mottled or vacuolated rarefactions and thinned cortices.

In many cases, however, the cortex becomes gravely weakened or even destroyed by the tumor tissue in one or several places of one or even of many bones. Thus, there is a bulge in the contour of the bone at such a site, and the tumor tissue which has spread out of the bone is found distending the periosteum but still restrained by it; or having violated the periosteum, it may even be found invading the local musculature. Exuberant growth of the myeloma at one bone site, with destruction of the cortex and spread of the tumor beyond the bone in this area, often

produces the lesion which first calls attention to the disease, though the marrow of the skeleton as a whole may already be riddled through by tumor tissue. For instance, a patient with multiple myeloma may first present himself because of a localized palpable and often painful enlargement of a rib, a clavicle, a jaw bone or even a long bone of an extremity —particularly a femur or a humerus. The patient showing such a lesion in a femur or a humerus may already have a pathologic fracture at the site of the presenting lesion (figs. 1 and 8).

However, it is remarkable how often one finds multiple myeloma presenting itself clinically because an exuberant focus of the disease has developed in a vertebral body (or several contiguous bodies). The affected body or bodies are found substantially destroyed; indeed, the tumor tends to transgress them, often producing pressure on the spinal cord or the local nerve trunks, with symptoms resulting (fig. 3 *C*). In such cases roentgenographic examination of the rest of the skeleton (including the skull) often fails to reveal the clearcut punched-out rarefactions which are conventionally held to distinguish the roentgenographic picture of multiple myeloma. It is in such cases (especially if one dorsal or lumbar body alone is clearly affected) that the true nature of the disease often goes unrecognized for long periods, the lesion frequently being interpreted as a local one, i.e., as a hemangioma, a giant cell tumor, a fracture due to Kümmell's disease or, if grossly destructive, as a focus of metastatic cancer (fig. 8 *B* and *C*). The true nature of the disease may become clear if marrow is obtained by sternal puncture or if serial roentgenograms of the rest of the skeleton finally demonstrate widespread lesions in other bones suggesting multiple myeloma, but sometimes it is discovered only at autopsy. Eventually, in the far advanced stage of the disease, the bones of the trunk and the limbs may become so extensively porotic and deformed from riddling by neoplastic tissue as to present the appearance of washed-out honeycomb bed shells roentgenographically. However, this extreme expression of skeletal devastation is observed only occasionally, even at autopsy (figs. 1 *A* and 2 *A*, *B* and *C*).

A somewhat special interest attaches to the calvarium. Great emphasis is usually laid on the diagnostic value of several or even many roundish punched-out rarefactions revealed in roentgenograms of the skull. It is true that when roentgenograms show clearcut and widespread involvement of the rest of the skeleton in the form of numerous punched-out rarefactions, the calvarium, too, is quite likely to show these, though not infrequently it fails to display them. But it is precisely when one turns to the roentgenograms of the calvarium because those of the other bones do not show the conventional picture of multiple myeloma that the calvarium, too, fails to show it. Whether or not the calvarium shows rarefactions, histologic examination will reveal that the marrow of the

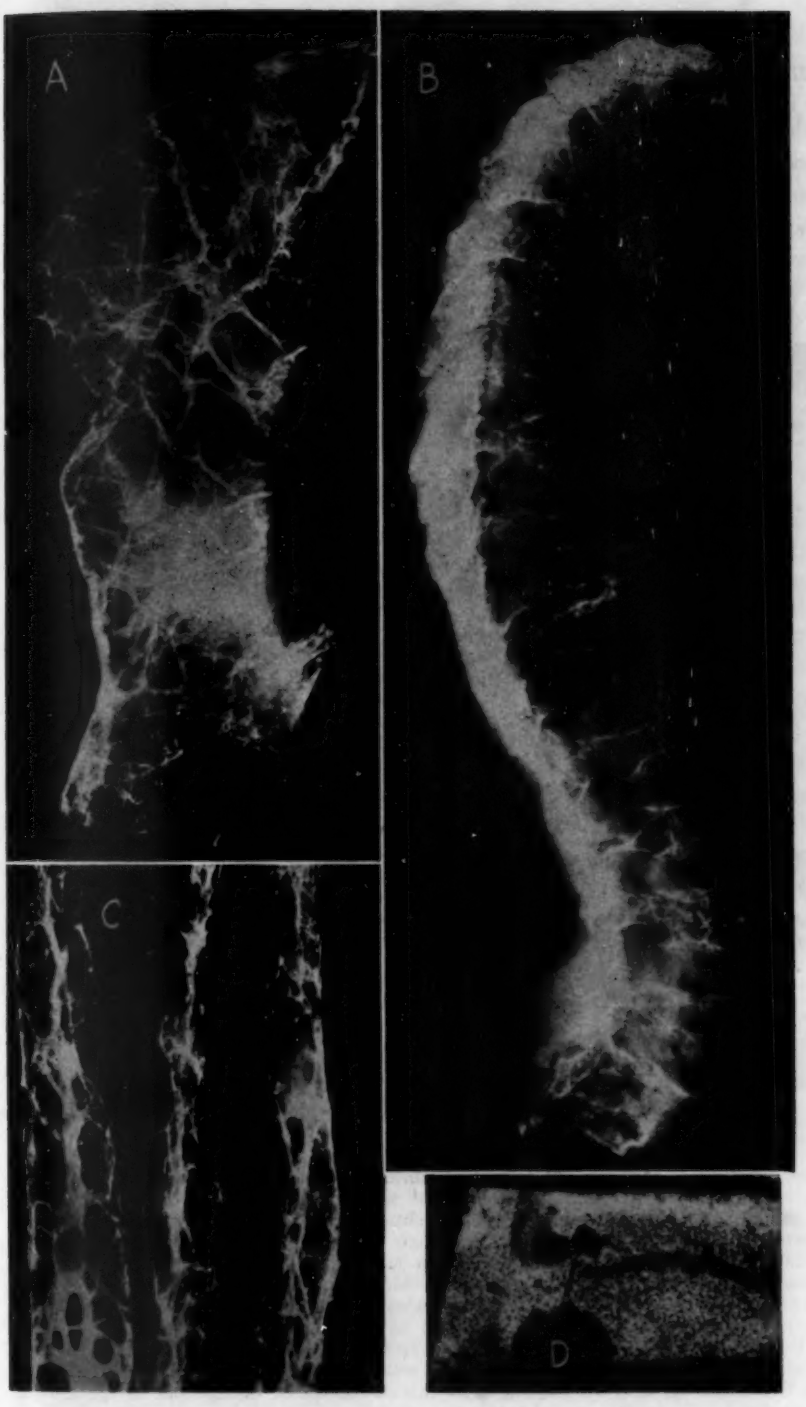

Fig. 2.—Roentgenograms of bone specimens obtained at autopsy illustrating the skeletal devastation that may be observed in far advanced multiple myeloma: *A*, an iliac bone showing a washed-out, honeycombed appearance, reflecting extreme porosity of the spongy bone and thinning of the cortex. *B*, vertebral column (same case) showing striking resorption of bone and compression of many of the vertebral bodies. *C*, several involved ribs (same case). *D*, a slice of the calvarium (a comparable case) showing a sharply punched-out defect in the tables. The involvement of the calvarium was considerably less pronounced than the involvement of the skeleton generally.

diploic spaces has been substantially replaced by the tumor tissue. At sites of clearcut rarefaction one will find that the tumor tissue is present as a nodule which has encroached on and destroyed the diploic bone, sometimes also thinning the tables, but in our experience the tables are seldom perforated even in such sites (fig. 7). Actually, the riddling of

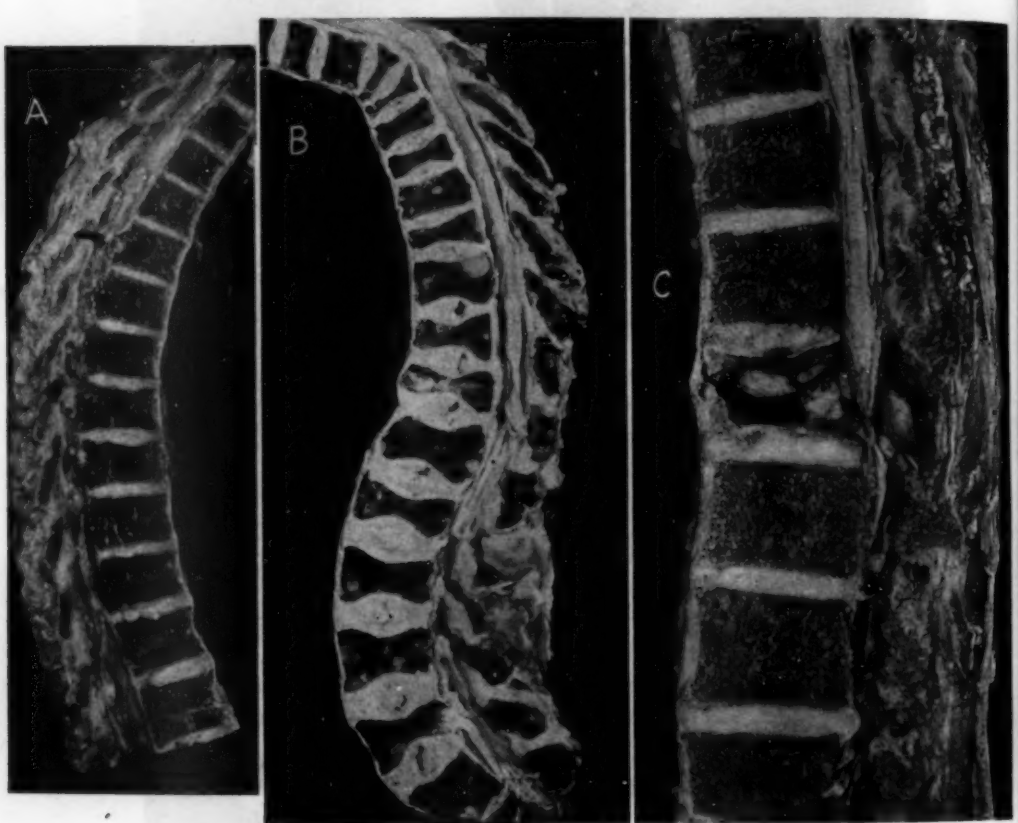

Fig. 3.—Specimens from several autopsies illustrating variations in the degree and the type of involvement of the vertebral column. *A* shows such inconspicuous lesions grossly that one has to search for scattered small nodes of myeloma tissue within the marrow of some of the vertebral bodies. *B* displays striking involvement. The vertebral bodies are extremely porotic and compressed; many of the intervertebral disks are correspondingly expanded; the marrow everywhere has been extensively replaced by myeloma, which also appears in the form of discrete tumor nodes; there are also tumor nodes of varying size within some of the spinous processes. The roentgenogram of this spine is pictured in figure 2 *B*. The skeletal changes in this case were associated with pronounced hypercalcemia and profound anemia. *C* shows almost total destruction and collapse of the body of the tenth dorsal vertebra and also extradural tumor encuffment of the cord at that level. The other vertebrae were not collapsed but showed miliary to small pea-sized grayish white areas of tumor tissue, some bodies presenting more of these than others. The compression of the spinal cord was followed by transverse myelitis and ascending infection of the urinary tract. This subject (a woman of 35) presented a leukemic blood picture terminally.

the calvarium by tumor deposits, which produce numerous circular punched-out defects, is not invariably an indication of multiple myeloma, since it sometimes occurs in carcinomatosis.[7]

HEMATOLOGIC OBSERVATIONS OF DIAGNOSTIC IMPORTANCE

In our cases the hematologic findings were essentially in agreement with those recorded in the literature, which point to the significant frequency with which anemia is encountered with multiple myeloma. The anemia reflects progressive neoplastic encroachment on the myeloid marrow, bleeding into tumor tissue and frequently also the effects of

Fig. 4.—Macerated specimen of the lower lumbar vertebral bodies and the innominate bones, showing moth-eaten erosions of these bones in places invaded by myeloma tissue.

general debilitation and renal damage. About 70 per cent of our patients presented appreciable reduction of hemoglobin and erythrocyte levels when first observed, and in about one half of them the anemia was already of moderate or severe grade. For example, a patient with far advanced skeletal involvement had a hemoglobin value on admission of only 4.2 Gm. per hundred cubic centimeters of blood and a red blood cell count of 1,400,000 per cubic millimeter. In any event, even if the anemia is not profound at first, the trend in most cases followed over a period of

7. Cosin, L.: Brit. J. Surg. **23**:110, 1935.

several or many months is toward slow but steady decline of the hemoglobin and erythrocyte values, often to appallingly low levels. Transfusions, even when repeated, appear to have merely a temporary sparing effect. In a notable case under observation for approximately a year, the hemoglobin value fell as low as 2.6 Gm. and the erythrocyte count to 1,000,0000 and remained at about these levels for six months until death resulted from cachexia, renal insufficiency and terminal bronchopneumonia. The profound anemia was reflected anatomically in intense hemosiderosis of liver, spleen, marrow and lymph nodes. Incidentally, the blood smears showed as many as 20 to 30 normoblasts per hundred white cells counted. The anemia of this particular patient was normocytic, as anemia usually is in cases of myeloma,[8] the color index being slightly below 1.0, but the anemia of some myelomatous patients may be macrocytic in type, simulating pernicious anemia.[9]

The depletion and irritation of the leukopoietic marrow in cases of myeloma is not infrequently reflected by the appearance in the blood smears of a few myelocytes or even myeloblasts, and occasionally these immature leukocytes may be present in such large numbers as to produce a leukemoid picture.[10] Thus, multiple myeloma, from a hematologic point of view at least, sometimes simulates myeloid leukemia at first, especially if a purpuric tendency is present. Sometimes, also, the number of eosinophils may be found increased as another indication of irritation of the marrow. The total leukocyte count shows no consistency and may be normal, somewhat depressed or slightly increased.

It is pertinent to mention also that any of the following phenomena may be observed in cases of multiple myeloma: excessive rouleau formation in blood smears, autohemagglutination of the red cells in dry and wet films, clumping with Hayem's solution, failure of clot retraction, abnormal viscosity of the blood and rapid sedimentation rate. It has been shown also that serums from myelomatous patients often have an anticomplementary property, although by no means all such serums give this reaction.[11] These phenomena in general have been ascribed to the presence of hyperproteinemia (which we shall discuss farther on), and their discovery may afford the first cue to the diagnosis of multiple myeloma.[12]

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8. Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1942, p. 631.

9. Sweigert, C. F.: Am. J. M. Sc. **190**:245, 1935. Astrua, P. C.: Minerva med. **1**:358, 1939.

10. Krumbhaar, E. B.: Am. J. M. Sc. **172**:519, 1926.

11. Jersild, M.: J. A. M. A. **113**:1119, 1939.

12. Foord, A. G.: Ann. Int. Med. **8**:1071, 1935. Foord, A. G., and Randall, L.: Am. J. Clin. Path. **5**:532, 1935. Jersild.[11]

Another important aid in the diagnosis of myeloma through hematologic methods is the finding of myeloma cells or so-called atypical plasma cells in blood smears. The frequency with which such cells are discovered and properly identified (beyond their provisional designation as "blast" cells) seems to depend largely on the thoroughness and skill of the observer,[13] and in suspected cases of myeloma blood smears should really be scrutinized by a qualified hematologist. They were discovered in only 2 of our cases on routine examination,[14] but Morissette and Watkins[15] claimed to have found them in as many as 41 of 56 cases studied. They pointed out that preliminary scrutiny of the smear under low magnification was necessary for the detection of these abnormal cells.

In most instances in which they are found, the myeloma cells are present in relatively small or, at most, moderate numbers, but occasionally there may be such an outpouring of them into the blood stream as to give rise to a frankly leukemic blood picture. Thus, in 1 of our cases (in which transverse myelitis was the cause of death) the leukocyte count during the terminal phase of the disease rose steadily from 6,000 to 40,000, and 30 to 54 per cent of the leukocytes were identified as plasma cells. Such cases of multiple myeloma in which the tumor cells readily invade the blood stream are well known and have been collected and discussed under the head of plasma cell leukemia by Muller and McNaughton,[16] Piney and Riach,[17] Osgood and Hunter,[18] Patek and Castle[19] and others. The pertinent cases coming to autopsy indicate conclusively that so-called plasma cell leukemia has its anatomic foundation in skeletal multiple myeloma and cannot logically be considered, as Osgood and Hunter have suggested, as a disease primarily of the blood. The development of "leukemia" in such cases represents a phase, and often a terminal phase, in the evolution of the disease. It is worth noting that cases of multiple myeloma characterized by frank invasion of the blood stream apparently exhibit a strong tendency toward extraskeletal spread in the form of diffuse and occasionally nodular infiltrations of the spleen, the liver and lymph nodes, and sometimes also of the kidney, the pancreas, the skin or other organs.

Still another valuable diagnostic aid has been advanced in recent years by hematologists, namely, the use of sternal marrow puncture. The

13. Mallory, T. B.: New England J. Med. **212**:28, 1935.
14. Rubin, G.: Bull. Hosp. Joint Dis. **3**:62, 1942.
15. Morissette, L., and Watkins, C. H.: Proc. Staff Meet., Mayo Clin. **17**: 433, 1942.
16. Muller, G.-L., and McNaughton, E.: Folia haemat. **46**:17, 1931.
17. Piney, A., and Riach, J. S.: Folia haemat. **46**:37, 1931.
18. Osgood, E. E., and Hunter, W. C.: Folia haemat. **52**:369, 1934.
19. Patek, A. J., and Castle, W. B.: Am. J. M. Sc. **191**:788, 1936.

experience of Rosenthal and Vogel,[20] Beizer and co-workers,[21] Waldenström [22] and others indicates that sternal puncture has high diagnostic value in cases of multiple myeloma, although it may not necessarily yield positive information in every instance.[22a] Certainly, it is a procedure that should be employed whenever the presence of myeloma is suspected, and especially when biopsy of some obviously affected bone is not feasible. In most instances of multiple myeloma, according to Wintrobe,[8] cellular marrow spreads will reveal myeloma cells making up 3 to 65 per cent of the total number of cells.

SIGNIFICANT BIOCHEMICAL CHANGES IN CASES OF MULTIPLE MYELOMA

Hypercalcemia.—The occurrence of hypercalcemia with multiple myeloma was noted [23] as early as 1927, while the observation that hypercalcuria and a negative calcium balance may be present dates back thirty years or more.[24] Hypercalcemia occurs in about one half of the cases of multiple myeloma, having been observed by us in 8 of the 16 cases investigated (in the remainder the calcium values were within the normal range). The increased calcium values ranged as high as 18 mg. per hundred cubic centimeters of serum. It should be noted that in such cases the kidneys tend to show deposits of calcium granules in the tubular epithelium and interstitial connective tissue. In some cases the metastatic calcifications may be quite heavy and widespread, involving, in addition, the interstitium of the lungs, the lining of the stomach and even other tissues.[25]

The increase of calcium in the serum reflects the lytic resorption of the bones and the tendency toward renal failure in many of the cases. It apparently develops independently of hyperproteinemia, since the highest calcium levels which we observed were in cases in which the serum protein concentration was normal. Apparently, in some instances the tendency toward hypercalcemia is perpetuated and accentuated by secondary hyperplasia of the parathyroid glands developing in response to chronic renal insufficiency. However, this mechanism does not invariably operate as Bulger and his co-workers believe.[26] Indeed, in our own

20. Rosenthal, N., and Vogel, P.: J. Mt. Sinai Hosp. **4**:1001, 1938.

21. Beizer, L. H.; Hall, B. E., and Giffin, H. Z.: Am. J. M. Sc. **203**:829, 1942.

22. Waldenström, J.: Acta chir. Scandinav. **87**:365, 1942.

22a. Aegerter, E., and Robbins, R.: Am. J. M. Sc. **213**:282, 1947.

23. Charlton, T. J.: Arch. Int. Med. **40**:98, 1927.

24. Williams, O. T.: Biochem. J. **5**:225, 1911. Blatherwick, N. R.: Am. J. M. Sc. **151**:432, 1916.

25. (*a*) Bender, O.: Deutsche Ztschr. f. Chir. **63**:370, 1902. (*b*) Froboese, C.: Virchows Arch. f. path. Anat. **222**:291, 1916.

26. Bulger, A. A.; Dixon, H. H.; Barr, D. P., and Schregardus, O.: J. Clin. Investigation **10**:143, 1930.

autopsies in which the parathyroid glands could be dissected out, these glands did not show significant enlargement.

Hypercalcemia per se is, to be sure, not diagnostic of multiple myeloma even when it is associated with rarefying skeletal lesions, since it also occurs characteristically with idiopathic hyperparathyroidism and occasionally with osteoclastic carcinoma extensively metastasizing to the skeleton. However, hypercalcemia associated with either hyperproteinemia or Bence Jones proteinuria is clearly indicative of multiple myeloma. It is precisely because of the presence of hypercalcemia, nephrocalcinosis and resorptive skeletal changes that some cases of multiple myeloma have been misinterpreted, at least temporarily, as instances of idiopathic hyperparathyroidism [27] and the patients even subjected to surgical exploration in a misguided search for a parathyroid adenoma.[28] Actually, as Jaffe [29] has pointed out, a proper appreciation of the roentgenographic appearance of the calvarium in cases of multiple myeloma should suffice to prevent confusion with hyperparathyroidism, irrespective of the roentgenographic changes elsewhere.

Furthermore, multiple myeloma is characterized by the fact that the serum phosphatase activity tends to be normal, no matter how extensive the skeletal involvement may be. It is true that if the serum phosphatase activity is measured shortly after the occurrence of a pathologic fracture, it may be found slightly increased, but the increase does not attain the level that is usually reached in advanced stages of hyperparathyroidism. In doubtful cases, in which the skeletal lesions are equivocal roentgenographically, the serum protein values are not significantly elevated and Bence Jones proteinuria is not detectable, marrow obtained by sternal puncture should also help to resolve the problem in differential diagnosis.

Hyperproteinemia.—One of the peculiar and characteristic features of some cases of myeloma is the presence of hyperproteinemia, specifically hyperglobulinemia. Indeed, the occurrence of abnormal protein in the blood of patients with multiple myeloma was noted as early as 1899 by Ellinger.[30] Hyperglobulinemia is observed in about half the cases of multiple myeloma; it was present in 8 of 16 cases investigated by us. Indeed, its incidence is claimed by some to run as high as 60 per cent. The globulin values as determined by the conventional Howe method in these cases ranged between 3 and 14 Gm. per hundred cubic centimeters of blood. The formaldehyde-gel and Takata reactions provide

27. Caylor, H. D., and Nickel, A. C.: Ann. Surg. **97**:823, 1933. Enzer, N., and Lieberman, B.: Ann. Int. Med. **8**:1062, 1935. Sager, W. W.; Choisser, R. M., and Weller, G. L., Jr.: J. Lab. & Clin. Med. **23**:1132, 1938.

28. North, J. P.: Am. J. Surg. **31**:563, 1936.

29. Jaffe, H. L.: Bull. New York Acad. Med. **16**:291, 1940.

30. Ellinger, A.: Deutsches Arch. f. klin. Med. **62**:254, 1899.

convenient crude tests for hyperglobulinemic serums,[31] but when positive they should be supplemented by precise quantitative methods. The serum albumin does not contribute to the hyperproteinemia, being normal, as a rule, when the globulin value is normal and actually diminished when the globulin value is increased. Thus, in cases of our series in which the serum globulins were normal in concentration the albumin values ranged between 4 and 5 Gm., while in 8 cases in which the globulin (and total protein) values were increased, the albumin values ranged between 2 and 3.5 Gm. The reason why the serum albumin values are consistently low in the cases of myeloma characterized by hyperglobulinemia seems to be that, in these cases particularly, damage of the renal tubules tends to develop, and thus the loss of albumin may conceivably be part of the complex of renal insufficiency.

Hyperglobulinemia per se is, of course, not necessarily indicative of multiple myeloma, since it is known to occur with other conditions also—particularly with chronic infections, notably lymphogranuloma venereum, sarcoidosis and kala-azar, and occasionally with cirrhosis of the liver, chronic nephritis, etc. However, hyperglobulinemia should always suggest the possibility of myeloma, and if found in association with hypercalcemia or Bence Jones proteinuria, speaks definitely for myeloma.

The protein composition of myeloma serums has been the subject of active investigation in recent years. Magnus-Levy,[32] who has written extensively on multiple myeloma, has advanced the view that the hyperproteinemia reflects exclusively an increase of the euglobulin and that the latter accounts for most of the physical aberrations of the blood commonly observed in cases of myeloma. We found the euglobulin significantly increased in only 3 of the 6 hyperglobulinemic serums investigated. In these 3 serums the high "euglobulin" ranged from 5 to 11 Gm. per hundred cubic centimeters and constituted 70 to 80 per cent of the total globulin, thus accounting for most, if not all, of the protein increment. However, in the other 3 serums the euglobulin levels were within the normal range.

Actually, Kekwick,[33] Devine [34] and Gutman, Moore, Kabat and their co-workers,[35] employing new physicochemical technics, have shown that the problems entailed in the separation, identification and quantitative estimation of the various normal and abnormal proteins which may be

31. Bing, J.: Acta med. Scandinav. **91**:336, 1937. Gutman, A. B., and Wise, C. R.: Proc. Soc. Exper. Biol. & Med. **35**:124, 1936. Waldenström.[22]

32. Magnus-Levy, A.: Ztschr. f. klin. Med. **126**:62, 1933.

33. Kekwick, R. A.: Biochem. J. **34**:1248, 1940.

34. Devine, J.: Biochem. J. **35**:433, 1941.

35. Gutman, A. B.; Moore, D. H.; Gutman, E. B.; McClellan, V., and Kabat, E. A.: J. Clin. Investigation **20**:765, 1941. Moore, D. H.; Kabat, E. A., and Gutman, A. B.: ibid. **22**:67, 1943.

present in the serums of myelomatous patients are extremely complex. The last-mentioned authors, in particular, have shown that there is extraordinary variability of the protein increment as determined not only by conventional fractional precipitation with neutral salts but also by improved electrophoretic technic (Tiselius apparatus), ultracentrifugation, protein solubility curves and immunologic methods. They have found that, apparently, the serums of most of the patients fall into one of three classifications: (1) serums of apparently normal composition with respect to serum proteins, (2) serums with hyperglobulinemia due to increase chiefly in the Howe euglobulin fraction and partly in the pseudoglobulin I fraction, comparable to what one may observe in chronic infections, and (3) serums with or without hyperglobulinemia giving a variety of anomalous patterns by Howe or electrophoretic methods, not encountered in other diseases, and apparently due for the most part to significant Bence Jones proteinemia. Their observations have led them to believe, furthermore, that Bence Jones protein, so called, is not a single substance but an ill defined group of similar proteins, thus confirming the observations of Bayne-Jones and Wright [36] based on immunologic reactions of Bence Jones proteins.[37]

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36. Bayne-Jones, S., and Wright, W. D.: Bull. Johns Hopkins Hosp. **33**:37, 1922.

37. The origin of these abnormal serum proteins, particularly of the Bence Jones proteins, has been the subject of much discussion. There is evidence at hand to indicate that serum proteins other than albumin and fibrinogen (which are believed to be synthesized in the liver) may originate in leukocytic cells of lymphoid or bone marrow origin. For example, gamma globulins concerned with antibody formation are believel to be derived from lymphoid cells (Dougherty, T. F.; Chase, J. H., and White, Proc. Soc. Exper. Biol. & Med. **57**:295, 1944; Kass, E. H.: Science **101**:337, 1945). More pertinent are the experiments of L. Meyler (Arch. Int. Med. **57**:708, 1936) on the nature of a Bence Jones-like protein substance extractible from normal marrow and also from leukocytes obtained from pus and leukemic tissue. These experiments suggest that small undetectable amounts of Bence Jones (or similar) protein normally derive from the sources indicated and that in multiple myeloma (and rarely also in certain other conditions affecting the marrow) the output is so magnified above the rate of destruction that the protein appears in the blood and is excreted as a pathologic constituent. Thus, there seems to be some justification for holding that the myeloma cells themselves, being derived presumably from leukopoietic marrow tissue, may elaborate and store abnormal proteins, and indeed the presence of a Bence Jones-like protein in myelomatous tissue has been demonstrated. On the other hand, there are those [34] who contend that the considerable amount of protein excreted in some cases of myeloma precludes the possibility of the protein's being a metabolite of the tumor cells and who hold that the protein in question results from a derangement of a synthetic mechanism in the marrow, which may result also in the formation of excessive euglobulin and of certain abnormal proteins, and possibly of amyloid substances as well (Boggs and Guthrie [43]; Magnus-Levy [32]).

This multiplicity of Bence Jones proteins and the difficulty of separating them from other normal and abnormal serum proteins make further progress in their identification and assay perplexing, although for the detection of small amounts of Bence Jones protein in serum the specific precipitin reaction devised by Hektoen [38] has been employed to advantage. Nor does the complexity of the problem end there. The observations of Wintrobe and Buell,[39] von Bonsdorff, Groth and Packalén [40] and Shapiro, Ross and Moore [41] indicate that the serums of myelomatous patients may on occasion contain still another peculiar abnormal protein substance of high molecular weight and great viscosity, differing from Bence Jones protein and showing a tendency to spontaneous precipitation and crystallization.

Bence Jones Proteinuria.—As is well known, the occurrence in the urine of protein giving the Bence Jones reaction is the earliest recorded observation in connection with the neoplasm now commonly designated multiple myeloma, dating back a century. It is also generally recognized that the presence of this reaction strongly favors the diagnosis of multiple myeloma (with certain reservations) but that, on the other hand, it may be absent in established cases of myeloma. We found evidence of Bence Jones protein in the urine in 10 of 26 cases investigated, although it has been stated [42] that it is found in at least 65 per cent of all cases of multiple myeloma. This discrepancy may be more apparent than real, and one must be persistent in searching for Bence Jones protein in the urine, since: (1) it may be undetectable in casual specimens but present in a twenty-four hour specimen, (2) it may be present at certain times but not at others (that is, its excretion may be intermittent rather than continuous) and (3) it may be absent early in the course of the disease and become evident later on. On the other hand, there are undoubtedly instances of myeloma in which no detectable Bence Jones protein is ever excreted. In 1 of our cases twenty-five examinations for Bence Jones proteinuria were made over a period of a year, all of them yielding negative results, including that made on urine taken from the bladder at autopsy. We have not observed any absolute correlation between the cytologic aspects of our myelomas and the incidence of Bence Jones proteinuria, although we do have the impression that the latter occurs somewhat more frequently with the relatively large cell myelomas

38. Hektoen, L.: J. A. M. A. **76**:929, 1921.

39. Wintrobe, M. M., and Buell, M. V.: Bull. Johns Hopkins Hosp. **52**:156, 1933.

40. von Bonsdorff, B.; Groth, H., and Packalén, T.: Folia haemat. **59**:184, 1938.

41. Shapiro, S.; Ross, V., and Moore, D. H.: J. Clin. Investigation **22**:137, 1943.

42. (*a*) Geschickter, C. F., and Copeland, M. M.: Arch. Surg. **16**:807, 1928. (*b*) Wintrobe and Buell.[39]

(characterized by hyperglobulinemia) than it does with the classic small cell plasmacytoma.

In regard to the specificity of Bence Jones proteinuria, it is universally stated that Bence Jones protein may occasionally be found in the urine in certain conditions other than multiple myeloma, especially in leukemia and in carcinoma metastatic to the skeleton. However, citations of actual experience to that effect are scarce in the literature. We have never encountered it in other conditions ourselves, although we have not made any systematic study of the Bence Jones reaction in skeletal diseases other than myeloma. There are a few pertinent observations, however—namely, those of Boggs and Guthrie,[43] Geschickter and Copeland [42a] and Bayrd and Heck [44]—purporting to show that rarely in chronic leukemia and metastatic carcinoma of bone and still more rarely in certain other diseases of bone (e. g., senile osteomalacia, gunshot wound, polycythemia) the urine may contain protein giving the Bence Jones reaction. With these exceptions, the finding of Bence Jones proteinuria is, for all practical purposes, indicative of multiple myeloma.

Increased Uric Acid in the Blood.—This represents still another significant, though not specific, change occurring with multiple myeloma. It has been commented on by Stewart and Weber [45] and has been observed by Tarr and Ferris [46] and also by us in several instances. In the opinion of Stewart and Weber, the uric acid increment results from the catabolism of nucleoproteins derived from the myeloma cells—an explanation that seems quite plausible. Multiple myeloma is, of course, not the only tumor responsible for hyperuricemia; it is well known, for example, that the latter may also occur with the leukemias.

EXTRASKELETAL MYELOMATOUS INFILTRATIONS

As noted, the extraskeletal occurrence of grossly discernible myelomatous foci is decidedly uncommon, although there can be no doubt that in occasional cases of multiple myeloma coming to autopsy (particularly in those in which the blood stream had been invaded by myeloma cells) one may find single or even multiple tumor foci within the internal organs. Microscopic infiltrations, particularly of the spleen, the liver or lymph nodes, are somewhat less unusual, but in our experience even these are lacking in most cases of multiple myeloma. Not infrequently, what may appear to be independent extraskeletal tumor foci occurring, for example, in the dura, the pituitary gland, the oropharynx and the nasopharynx, the larynx, the thyroid gland, the pleura and the retroperitoneal, mediastinal and subcutaneous tissues may actually represent direct outgrowths

43. Boggs, T. R., and Guthrie, C. G.: Bull. Johns Hopkins Hosp. **23**:353, 1912.
44. Bayrd, E. D., and Heck, F. J.: J. A. M. A. **133**:147, 1947.
45. Stewart, A., and Weber, F. P.: Quart. J. Med. **7**:211, 1938.
46. Tarr, L., and Ferris, H. W.: Arch. Int. Med. **64**:820, 1939.

of tumor of contiguous bones. Also, in evaluating the reported cases of supposed multiple myeloma characterized by extensive and widespread tumor deposits in the internal organs, it is important to bear in mind that some of them may actually have been instances of metastasizing occult carcinoma,[47] malignant lymphoma or stem cell leukemia.

Nevertheless, cases of myeloma demonstrating the presence of extraskeletal tumor foci are prominently featured in the literature precisely because of their unusual nature, and infiltration of practically any organ that might be named has been noted at one time or another. Thus, Mallory [48] has cited a case of multiple myeloma in which a single metastatic nodule was found in a lung (and also in a bronchial lymph node), and similar instances of pulmonary metastasis have been noted also by Hallermann,[49] Carlisle,[50] Piney and Riach [17] and Batts.[4] Metastases have been observed in the heart by Piney and Riach and by Carlisle, who commented on the finding of two discrete bean-sized nodules of myeloma within the wall of the right auricle. Nodular infiltrations of an enlarged spleen have been observed by Osgood and Hunter,[18] and Churg and Gordon [51] have described a remarkable case in which the spleen (weighing 650 Gm.) presented innumerable tumor nodules studding and substantially replacing the pulp. In the latter case, infiltration of some of the intra-abdominal lymph nodes and of the portal areas and sinusoids of the liver was also noted. Piney and Riach have described the finding of a tumor node, the size of a plum, within the pancreas, and Patek and Castle,[19] with regard to a case exhibiting "plasma cell leukemia," commented on the observation of a soft, reddish brown tumor node, 1.5 cm. in diameter, in the tail of the pancreas. In the latter case, incidentally, infiltration of the liver, the spleen, the kidneys and the abdominal lymph nodes was also noted microscopically. A metastasis in the form of a cherry-sized tumor node has been observed in an adrenal gland by Piney and Riach, and adrenal metastases have been noted by others. Involvement of the kidney has been noted by Carlisle, Morse,[52] Donhauser and DeRouville [53] and by Newns and Edwards,[54] the last describing the finding of an essentially circumscribed rounded tumor, 4 cm. in diameter, replacing part of the lower pole of a kidney and bulging into its pelvis. Involvement of the tonsils was noted in an extraordinary case reported by Jackson, Parker and

47. Nicholls, A. G.: Canad. M. A. J. **17**:301, 1927.
48. Mallory, T. B.: New England J. Med. **215**:1133, 1936.
49. Hallermann, W.: Deutsches Arch. f. klin. Med. **165**:57, 1929.
50. Carlisle, V.: South African M. J. **12**:298, 1938.
51. Churg, J., and Gordon, A. J.: Arch. Path. **34**:546, 1942.
52. Morse, P. F.: J. Cancer Research **5**:345, 1920.
53. Donhauser, J. L., and DeRouville, W. H.: Arch. Surg. **43**:946, 1941.
54. Newns, G. R., and Edwards, J. L.: J. Path. & Bact. **56**:259. 1944.

Bethea,[55] in which a "plasmacytoma" in that region had been removed fully eight years before generalized involvement of bone could be detected, although the neoplasm had extended to the cervical lymph nodes in the interim. Striking involvement of the intestinal tract has been described by Carlisle,[50] who found numerous tumor deposits in the submucosa of the duodenum and small bowel, the mucosa being tautly stretched over these protuberances. Ovarian metastases have been noted by Herrick and Hektoen,[56] and bilateral tumorous involvement of the male gonads has been observed by Ulrich.[57] Cutaneous involvement in the form of multiple raised nodular infiltrations (scattered over the scalp, the region of the clavicle and the arm) has been described by Duvoir and associates,[58] and Kin [59] has reported a comparable case in which the nodular infiltrations (over the face and the back) were large, umbilicated and ulcerated, presenting the general picture of so-called mycosis fungoides. Piney and Riach [17] have described a most unusual case of multiple myeloma with "leukemia" in which the subject presented a diffuse nodular eruption over the trunk and limbs, looking not unlike neurofibromatosis clinically, and also a peculiar helmet-like thickening extending down from the infiltrated scalp of the region of the forehead, in front of the ears and down to the nape of the neck. Incidentally, in this case, as in some of the others characterized by a leukemic blood picture, histologic examination showed infiltration of multiple sites, including the cervical lymph nodes, the kidney, the liver and the heart.

In our own autopsy material we encountered extraskeletal tumor foci only twice. In one instance (in which myeloma cells had been detected in blood smears during life) we encountered a solitary circumscribed whitish nodular focus of myeloma, about 0.5 cm. in diameter, within the liver subcapsularly. In this case we also observed collections of myeloma cells within the sinuses of the spleen on microscopic examination. In the other case, the lymph nodes along the aorta, within the mesentery, at the hilus of the liver and that of the spleen and around the pancreas were all more or less distinctly enlarged by sheets of tumor cells replacing the normal structure. The liver and the spleen were likewise appreciably enlarged and were seen microscopically to be infiltrated by collections of similar cells. Indeed, the possibility of aleukemic lymphadenosis was at first considered despite a positive Bence Jones reaction, but the observation that the bone marrow was diffusely

55. Jackson, H., Jr.; Parker, F., Jr., and Bethea, J. M.: Am. J. M. Sc. **181**: 169, 1931.

56. Herrick, J. B., and Hektoen, L.: M. News **65**:239, 1894.

57. Ulrich, H.: Arch. Int. Med. **64**:994, 1939.

58. Duvoir, M.; Pollet, L.; Layani, F.; Dechaune, M., and Gaultier, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **54**:687, 1938.

59. Kin, S. S.: Arch. f. jap. Chir. **16**:79, 1939.

infiltrated by cells resembling myeloma cells and the finding of renal changes characteristic of multiple myeloma established the latter diagnosis unequivocally.

Whether myeloma cell infiltrations of the liver, the spleen or lymph nodes represent hematogenous metastases of tumor occurring in the bones or, rather, tumor foci independently formed as a result of activation and neoplasia of potential blood-forming cells within the organs in question does not concern us here directly. Sometimes infiltration of the spleen and the liver may already be manifested grossly by whitish streaks or nodules, or in the case of lymph nodes, by diffuse enlargement and cellularity, but more often it is detected only on microscopic examination. On the other hand, our experience definitely runs counter to that of Lowenhaupt,[60] who claimed to have found "plasma cells" of myelomatous nature in every one of the spleens from 12 patients who came to autopsy, in all of the lymph nodes examined and in three of the livers as well. It should be pointed out, however, that the indubitable identification of isolated small nests of cells presumed to be myeloma cells and their clearcut differentiation from cells of histiocytic or inflammatory nature commonly encountered in the spleen and the lymph nodes, particularly, may be exceedingly difficult. Furthermore, as Mallory [61] has rightly emphasized, small collections of immature blood cells representing foci of compensatory extramedullary hemopoiesis are observed rather frequently in cases of multiple myeloma, and these may also be mistaken for nests of myeloma cells.

SIGNIFICANT RENAL CHANGES IN CASES OF MULTIPLE MYELOMA

In the patient with multiple myeloma there are certain cytologic renal changes of almost pathognomonic distinctiveness. These changes may be manifested clinically in more or less heavy albuminuria, though not necessarily Bence Jones proteinuria, and in diminished power of the kidney to concentrate and to clear nitrogenous constituents. Only exceptionally do they result in the development of edema or hypertension. It has been emphasized on that account (Foord and others) that the finding of "atypical nephritis" should always suggest the possibility of multiple myeloma. When the renal damage is limited in extent, it results clinically in no more than persistent albuminuria. On the other hand, when it is more severe, it often leads ultimately to progressive renal insufficiency. Occasionally it leads to the relatively early appearance of azotemia, which dominates the clinical picture before the presence of multiple myeloma is even suspected.[62]

60. Lowenhaupt, E.: Am. J. Path. **21**:171, 1945.
61. Mallory, T. B.: New England J. Med. **224**:559, 1941.
62. McDonald, R. H.: Cleveland Clin. Quart. **10**:36, 1943. Thannhauser, S. J., and Brereton, H.: Bull. New England M. Center **4**:99, 1942.

Already in 1921, Löhlein [63] noted the deposition of crystalloid hyalin-like casts in the renal tubules of a subject with multiple myeloma coming to autopsy. Many of the casts plugging the tubules were surrounded by polymorphonuclear leukocytes, and often also by giant cells, which Löhlein thought were derived from proliferated tubular epithelium. There was some dilatation of the tubules, also some interstitial scarring, but the glomeruli and the blood vessels were not remarkable. The specificity of these essential changes was confirmed by Perla and Hutner,[64] Ehrich,[65] Bell,[66] Fishberg,[67] Forbus and co-workers,[68] Morison,[69] Blackman and his co-workers,[70] Newns and Edwards [54] and others. Indeed, Mallory [71] and his colleagues spoke of the "myeloma kidney" and maintained that it is often possible to make a diagnosis of multiple myeloma from looking at the kidney sections alone.

Grossly, in our 18 autopsies the kidneys were usually not remarkably altered. In 2 instances, however, they were found to be markedly contracted as a result of extensive interstitial scarring. On reviewing the sections of kidneys obtained in the autopsies we, too, were impressed by the constant finding of rather dense eosinophilic hyalin-like plugs of proteinaceous material in the renal tubules, particularly in the lower portions of the nephrons. In about half of the kidneys examined, one could find foreign body giant cells or clumps of polymorphonuclear leukocytes or both about the casts, although at times one had to search for them (fig. 5). In some instances the proteinaceous plugs were rather scattered, but in others they were numerous. It is pertinent in this connection to recall the exceptional case reported by Holman, in which the tubular obstruction was extensive enough to result in complete urinary suppression. It is noteworthy that the characteristic tubular plugs were found by us in cases in which the Bence Jones reaction had been negative as well as in those in which it had been positive. It seems altogether probable that abnormal proteins other than Bence Jones protein are also precipitated out in the tubules. We observed no significant glomerular changes in our material, although it may be noted in passing that Foord [12] emphasized the possible role of

63. Löhlein, M.: Beitr. z. path. Anat. u. z. allg. Path. **69**:295, 1921.

64. Perla, D., and Hutner, L.: Am. J. Path. **6**:285, 1930.

65. Ehrich, W.: Ztschr. f. klin. Med. **121**:396, 1932.

66. Bell, E. T.: Am. J. Path. **9**:393, 1933.

67. Fishberg, A. M.: Hypertension and Nephritis, ed. 4, Philadelphia, Lea & Febiger, 1939, p. 369.

68. Forbus, W. D.; Perlzweig, W. H.; Parfentjev, I. A., and Burwell, J. C., Jr.: Bull. Johns Hopkins Hosp. **57**:47, 1935.

69. Morison, J. E.: J. Path. & Bact. **53**:403, 1941.

70. Blackman, S. S., Jr.; Barker, W. H.; Buell, M. V., and Davis, B. D.: J. Clin. Investigation **23**:163, 1944.

71. Mallory, T. B.: New England J. Med. **221**:983, 1939.

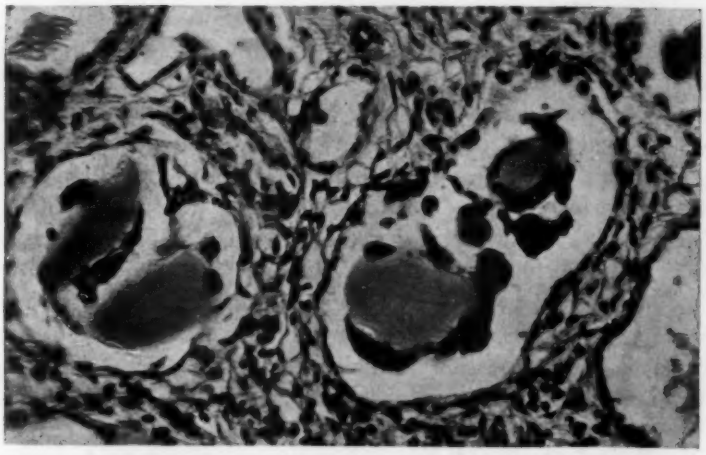

Fig. 5.—Photomicrograph of a section of kidney from a case of multiple myeloma, showing tubules obstructed by proteinaceous casts, about which there is a conspicuous foreign body giant cell reaction. × 200.

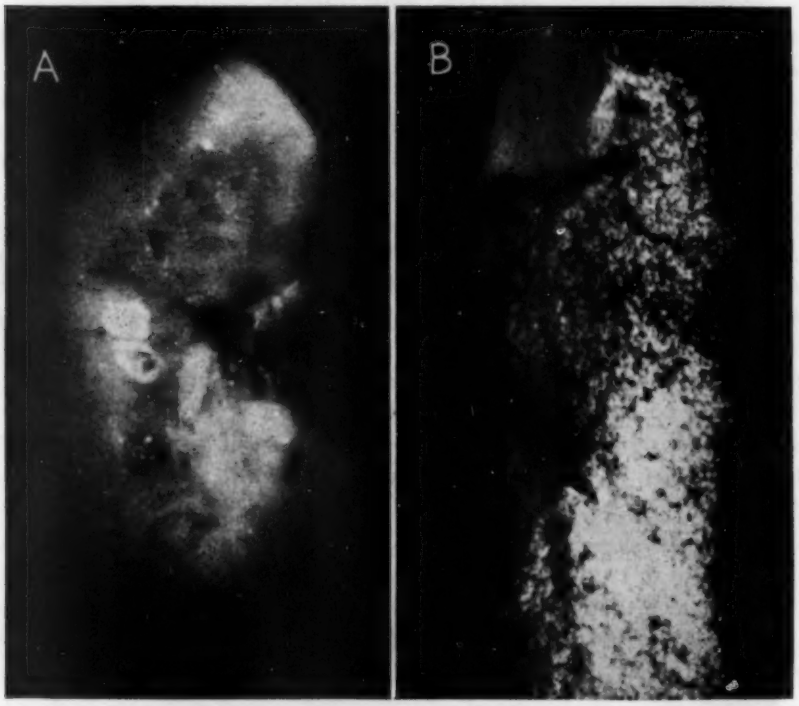

Fig. 6.—Roentgenograms from a case of multiple myeloma (the one pictured in figs. 2 *A*, *B* and *C* and 3 *B*) illustrating metastatic calcification. *A*, kidney showing calcareous material deposited within some of the calices (calcific gravel was present also in the urinary bladder). *B*, lung showing heavy calcification, principally of the lower lobe. The subject (a man of 32), toward the end of his course, presented extreme demineralization of the skeleton, hypercalcemia (17.8 mg. of calcium per hundred cubic centimeters of serum) and mounting renal insufficiency.

obstruction of glomerular capillaries by highly concentrated protein or by clumped erythrocytes as a factor in the development of renal insufficiency. We did, however, observe degenerative changes in the tubular epithelium in a number of the kidneys, as evidenced by the granular, vacuolar or hyaline droplet appearance of the lining cells and a tendency toward desquamation.

Some of the kidneys also exhibited a number of other changes worthy of mention. In several, slight to moderate arteriosclerotic contraction was noted (which must be evaluated in the light of the fact that these subjects were older adults). In one kidney pyelonephritis was present, having followed on compression of the spinal cord and ascending infection of the urinary tract. In another what appeared to be amyloid in granular or droplet form was present within the tubular epithelium and lumens, though not in the glomeruli. In one (in a case of far advanced myeloma featured by rapidly progressing, extreme demineralization of the skeleton and hypercalcemia, the calcium amounting to almost 18 mg.) evidence of deposition of calcium was observed grossly as well as microscopically (fig. 6 *A*). In this kidney (there was also heavy metastatic calcification of the lungs [fig. 6 *B*]) one could recognize yellowish streaks within some of the pyramids, and fine yellowish calcific gravel was present in many of the calices and within the urinary bladder as well.

AMYLOIDOSIS IN RELATION TO MULTIPLE MYELOMA

Another arresting feature of the pathologic anatomy of multiple myeloma is the presence of amyloid in some cases. Atkinson [72] found amyloidosis complicating myeloma in 40 of 643 cases of myeloma collected from the literature. On this basis the incidence is about 6 per cent. In our own material it was about 10 per cent. The presence of amyloid, particularly since it seems to occur in association with hyperglobulinemia and Bence Jones proteinuria, affords ground for speculation as to the chemical relations which may exist between the abnormal blood proteins and the amyloid protein substances. It has been suggested [73] that the abnormal blood proteins may serve as the mother substance of the amyloid proteins and that the amount and the distribution of the amyloid are determined also by disturbed fibroblastic activity.[74] Whatever the mechanism of amyloid formation may be in the body in general, it does seem probable that excessive formation of abnormal globulins is an essential condition for the deposition of amyloid, and it is conceivable that the latter represents a reaction to the foreign proteins in question.

72. Atkinson, F. R. B.: M. Press **195**:312 and 327, 1937.
73. Magnus-Levy.[32] Tarr and Ferris.[46]
74. Warren, S.: Am. J. Path. **6**:161, 1930.

So far as the skeleton is concerned, if amyloid is found, it usually appears in the form of scattered deposits detectable only microscopically in the neoplastic tissue of the affected bones (fig. 7). In an occasional case,[75] however, one finds not only the microscopic deposits but large agglomerations of amyloid intermingled with and substantially replacing myelomatous foci. In Freund's [75a] case the amyloid took the form of multiple calcifying amyloid tumors, one of which, breaking out of the seventh dorsal vertebral body, had produced extradural compression of the cord. The presence of multiple myeloma as a basis for the amyloid tumors in this case was recognized only on microscopic examination, there having been no grossly discernible tumor nodes within the bones but rather a diffuse infiltration of the marrow. In this case the amyloid was entirely limited to the skeleton. On the other hand, we have observed a case of so-called atypical amyloidosis in which autopsy failed to reveal evidence of amyloid in the myelomatous tissue itself, though practically

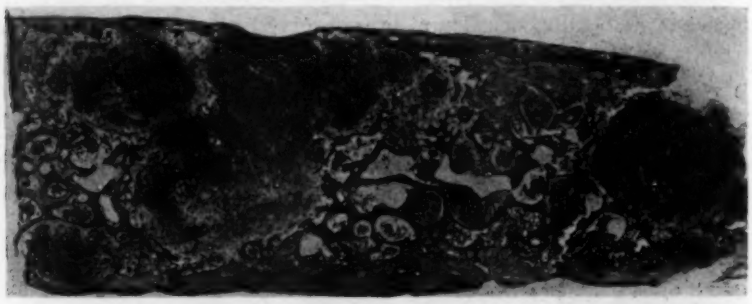

Fig. 7.—Photomicrograph of a cross section of the calvarium from a case of multiple myeloma, showing nodular foci of amyloid intermingled with myeloma cells. The outer table of the calvarium is completely eroded in one area. × 6.

all the extraskeletal tissues and organs, including the voluntary muscles and the skin, were heavily infiltrated with amyloid.

In general, one of the striking features of amyloidosis appearing in association with multiple myeloma is the frequency with which amyloid is deposited in unusual sites, either more or less diffusely or in the form of tumor-like masses which may attain large bulk. In contrast to the commonplace amyloidosis of parenchymatous organs (particularly the liver, the spleen, the adrenal glands and the kidneys), one may find amyloid deposited in great quantity in bones, in muscles, in joint capsules and in the skeletal connective tissues generally, in the skin and subcutaneous tissue, in the buccal and anal mucous membranes, in the tongue, in the heart, in the lungs, in the intestines, in the genitourinary tract

75. (*a*) Freund, E.: Frankfurt. Ztschr. f. Path. **40**:400, 1930. (*b*) Stadler, L.: Folia haemat. **61**:353, 1939. (*c*) Rosenblum, A. H., and Kirshbaum, J. D.: J. A. M. A. **106**:988, 1936.

and in other tissues. Indeed, so often is multiple myeloma the basis for so-called atypical or idiopathic amyloidosis that the possiblity of myeloma should be investigated in every case, even though the bones present no apparent evidence of tumor either roentgenographically or on gross inspection at autopsy.

These cases of unusual amyloidosis are all of great interest, but we may single out certain ones for special comment. In 1 of our cases (the one mentioned on page 28) involvement of the skin and subcutis led to remarkable scleroderma-like thickening and the occurrence of amyloid-containing verrucae, which were found on the eyelids, about the anus, on the oral mucous membrane and along the margins of the enlarged, rubbery, ulcerated tongue. Also noteworthy are the cases in which the mucosa or the muscular coat of the small intestine is extensively infiltrated by amyloid, since this deposition may lead at times to clinically puzzling intestinal obstructon.[76] Of particular interest, also, are the cases standing out because of the presence of multiple, smaller or larger, localized amyloid masses attached to the periosteum of the bones, especially near their articulations, and situated also in the skeletal muscles of the trunk and the extremities and about and within joint capsules. In relation to the latter, the amyloid may extend to the synovium and sublining tissue and, at times, even erupt into the joints.[77] Among the regions that may be selected are the hands, the antecubital fossae, the shoulders and the articulations of the clavicle. The presence of such joint swellings, associated with pain and limitation of motion, sometimes leads to a clinical diagnosis of rheumatoid arthritis, which may be entertained for years before the nature of the condition is recognized.[46] The amyloid masses seen in these cases are described as having a firm, grayish yellow or pinkish, lardaceous or glassy appearance somewhat suggestive of the flesh of fish, and microscopically they present as amorphous, poorly cellular, generally eosinophilic aggregates, about which one may observe foreign body giant cells. It is noteworthy also that this amyloid material, either in part or throughout, may fail to give the usual metachromatic staining reactions with one or another, or perhaps all, of the dyes commony employed for the detection of amyloid.

RELATIONSHIP OF APPARENTLY SOLITARY MYELOMA AND MULTIPLE MYELOMA

As has been stated, there are occasional cases of myeloma in which the first skeletal manifestation is that of an exuberant tumor focus within some one bone (commonly a femur or a humerus, but sometimes a vertebral body, an innominate bone, a bone of the calvarium or some other bone) and in which clearcut roentgenographic evidence of impli-

76. Randall, O. S.: Am. J. Cancer **19**:838, 1933. Bell.[66]

77. Hueter, C.: Beitr. z. path. Anat. u. z. allg. Path. **49**:100, 1910. Paige, B.: Am. J. Path. **7**:691, 1931. Stewart and Weber.[45] Stadler.[73b] Tarr and Ferris.[46]

cation of other bones does not appear for a number of years. We have observed 2 such cases. In one of them, attention was at first centered on an apparently solitary neoplastic focus in the third lumbar vertebral body (fig. 8 *B* and *C*). Indeed, this lesion was irradiated as a possible hemangioma or giant cell tumor. It was not until two and a half years after the onset of symptoms that a veritable shower of foci of myeloma appeared throughout the skeleton. In the other case the presenting focus (identified by biopsy as a myeloma) was a large tumor apparently localized within the upper end of the right femur. This tumor had transformed the upper part of the shaft, the intertrochanteric region and the neck into a ballooned-out, rarefied and coarsely honeycombed lesion simulating a peculiar cyst or giant cell tumor (fig. 8 *D*). Roentgenograms of the pelvis and the upper ends of the femurs (the only roentgenograms taken at the time) showed, otherwise, merely equivocal involvement of the right ilium and suggestive rarefaction shadows within the greater trochanter region of the left femur. This patient received radiation therapy at another hospital, with clinical improvement resulting. The case was subsequently included among those reported by Coley,[78] who likewise interpreted it as one of solitary myeloma. From him we learned that the patient died after a terminal wasting illness suggesting dissemination of tumor, but not until she had survived almost ten years after the onset of complaints referable to the tumor of the femur. Many other cases of supposedly solitary myeloma have been reported, including some with entirely inadequate follow-up records; they attracted attention [79] because they tended to controvert the conventionally doleful prognosis for myeloma in general. Among the more detailed and informative of these reports are those of Bailey,[6a] Pasternack and Waugh [6b] and Stewart and Taylor,[6c] citing survivals of seven, seven and a half and eight years, respectively, in patients who were still alive and apparently in good health at the time of publication. The consensus [79b] with regard to treatment seems to be that roentgen therapy has considerable value in affording relief from pain and possibly also in retarding local growth and eventual dissemination of the tumor.

In regard to these initially localized myelomas, it is hardly possible to determine from the available evidence whether the tumor in any given instance was entirely confined to the skeletal focus first attracting attention or whether it was already present elsewhere in the skeleton but clinically and roentgenographically silent. It has been claimed by the advocates of the former view that the negative result of roentgenographic examination of the remainder of the skeleton affords proof of the solitary nature of the myeloma in question. However, it is well known that the marrow throughout the skeleton may be extensively

78. Coley, W. B.: Ann. Surg. **93**:77, 1931.

79. (*a*) Cutler, M.; Buschke, F., and Cantril, S. T.: Surg., Gynec. & Obst. **62**:918, 1936. (*b*) Gootnick, L. T.: Radiology **45**:385, 1945.

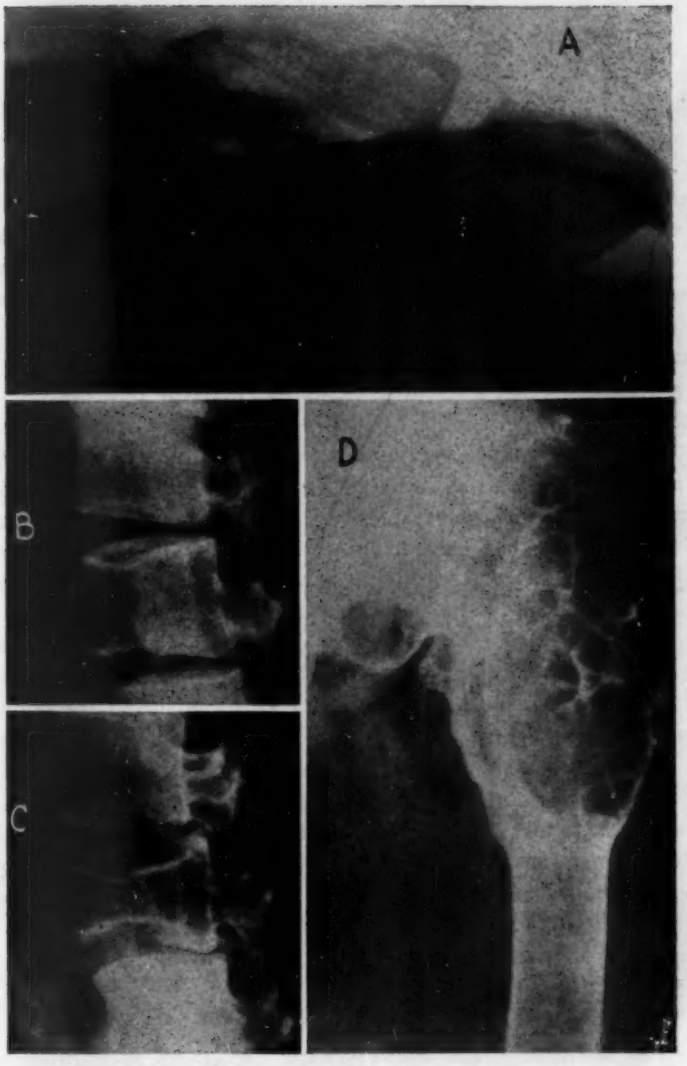

Fig. 8.—Roentgenograms of a number of myelomas from cases in which the tumor appeared initially to be localized in some one bone but subsequently showed obvious spread over the skeleton. Such tumors are usually not recognized as myelomas prior to biopsy, being commonly mistaken for giant cell tumor, hemangioma, bone cyst and other types of growth.

A, a myeloma of the acromial end of a clavicle, which presents an expanded multilocular appearance. A fracture extends through the sternal extremity of the expanded area. At the time no clearcut foci of myeloma were discernible elsewhere, but roentgenographic examination almost three years later revealed the presence of a single small punched-out defect in the calvarium and two additional foci in the femurs. Also, marrow obtained by puncture of the sternum showed nests of myeloma cells interspersed among the normal constituents of the marrow.

B, a myeloma appearing initially in the third lumbar vertebral body and apparently confined to that bone.

C, the same lesion as it appeared subsequently; the affected body has collapsed in spite of radiation therapy. About two and a half years after the onset of symptoms a shower of myelomatous foci appeared throughout the skeleton.

D, another large, curiously expanded myeloma apparently localized within the upper end of a femur. In this case, also, there was eventual dissemination of the tumor, but the subject survived almost ten years after the onset of complaints.

permeated by myeloma without this being evident roentgenographically. The long period of latency is also cited as proof, but, as we have seen, there is no certainty, when dealing with such a tumor, that foci of myeloma may not appear throughout the skeleton at any time. Indeed, as such cases are followed the number of survivors falls off steadily from year to year, so that at the end of a ten year period of observation few ostensibly solitary myelomas are left.[79] Also cited as proof of actual one bone localization is the absence of anemia and of Bence Jones proteinuria, but these are hardly trustworthy indications.

A more valid criterion would be sternal marrow punctates showing absence of myeloma cells—a test which was resorted to in few of the cases held to represent cases of genuine solitary myeloma[80]—but even this is not infallible. When one turns to the pertinent autopsy reports in the literature for more satisfactory proof of the existence of genuine solitary myeloma, one finds that there have been few in which the skeleton was examined with sufficient thoroughness both grossly and microscopically to exclude convincingly the possibility that myeloma occurred in other bones. It is true that on the basis of cases such as those reported by Harding and Kimball[81] and by Rutishauser[82] one has to admit the possibility of there being a genuine solitary myeloma, but the condition, if it actually occurs, must be quite rare. All one can say with certainty is that there are occasional cases in which myeloma starts out by producing an exuberant tumor focus in some one bone, commonly a long bone, and then tends to remain latent (in spite of pathologic fractures) for a long time, sometimes even as long as ten years, without giving rise to clearly discernible foci in other bones. The great likelihood is that in such cases the myeloma, if followed for a sufficiently long time, will eventually show obvious dissemination.

CYTOLOGIC CHARACTER OF MULTIPLE MYELOMA

The tumor tissue in multiple myeloma (when its appearance is not modified by hemorrhage, degeneration and necrosis, fracture of the bone or extension of the tumor into the soft parts) tends characteristically to be composed of large aggregates or veritable sheets of more or less compacted cells without any discernible intercellular material and without conspicuous supporting stroma. However, where the bone marrow is being invaded by tumor one observes marrow cells intermingled with tumor cells which, as they proliferate, tend to crowd out and eventually replace the marrow constituents. In selecting material for sectioning, one should choose some blocks of solid tumor tissue (relatively free of secondary changes) which do not require decalcification, since treatment

80. Bichel, J., and Kirketerp, P.: Acta radiol. **19**:487, 1938.
81. Harding, W. G., II, and Kimball, T. S.: Am. J. Cancer **16**:1184, 1932.
82. Rutishauser, E.: Centralbl. f. allg. Path. u. path. Anat. **58**:355, 1933.

with acid tends to shrink the cells and to darken and obscure nuclear detail. Most of our histologic preparations were stained with hematoxylin and eosin, though some were dyed with eosin-methylene blue, which seems particularly well suited to bringing out cytologic detail.

It has been recognized and must be emphasized that the cytologic picture is not the same in all specimens of multiple myeloma. Roughly, however, the tumors can be fitted into two general cytologic groups. On the one hand, there are those in which the tumor cells are quite uniform and predominantly small, and have a superficial resemblance to plasma cells. The tumor cell is roundish and has a stippled nucleus substantially filling the cell. The darkish chromatin particles spotting the nucleus are dispersed centrally as well as peripherally, and one actually observes nothing resembling a cart wheel in the sense of spokes radiating from a hub. The cytoplasm tends to be uniformly eosinophilic, though in occasional tumors one may observe lighter-staining perinuclear demilunes. Interspersed among these cells there may be some cells which, though of the same general character, are larger in respect to both cytoplasm and nucleus. There may also be occasional cells with double nuclei, but there is no tendency to cellular irregularity otherwise. It is to the myeloma showing this cytologic character that the name "plasma cell myeloma" or "plasmacytoma" is commonly applied (although these names have come to be rather indiscriminately applied to most myelomas, even to those in which the tumor cells have only the remotest resemblance to plasma cells) (fig. 9 *A*).

In the other group of myelomas the cytologic picture tends to be dominated by cells larger than those resembling plasma cells, but may be a rather variegated one. The dominant cells in the tumor generally exceed the myeloblast in size and, on the whole, show fairly abundant cytoplasm and have a large, round, oval or even reniform, pale stippled nucleus. The latter is not necessarily eccentric and indeed is often centrally placed. In some tumors the nuclei of certain of the cells may contain a well defined pinkish or reddish round body resembling a nucleolus. The cytoplasm is generally eosinophilic but sometimes takes a more basophilic or polychromatic tinctorial hue, and in some tumors it also presents paler demilunes around the central face of the nucleus. Occasionally the cytoplasm is vacuolated or contains refractile rod-shaped bodies, considered by some to be of protein nature.[83] In any particular tumor site examined, one may also find some of the smaller cells resembling plasm cells or, on the other hand, find cells which are much larger than the dominant ones and frequently show nuclear atypism. Specifically, such atypical cells may present large and hyperchromatic nuclei of bizarre shape, or two or more nuclei (fig. 9 *B* and *C*; fig. 10 *A*, *B* and *C*).

83. Steinmann, B.: Deutsches Arch. f. klin. Med. **185**:49, 1939.

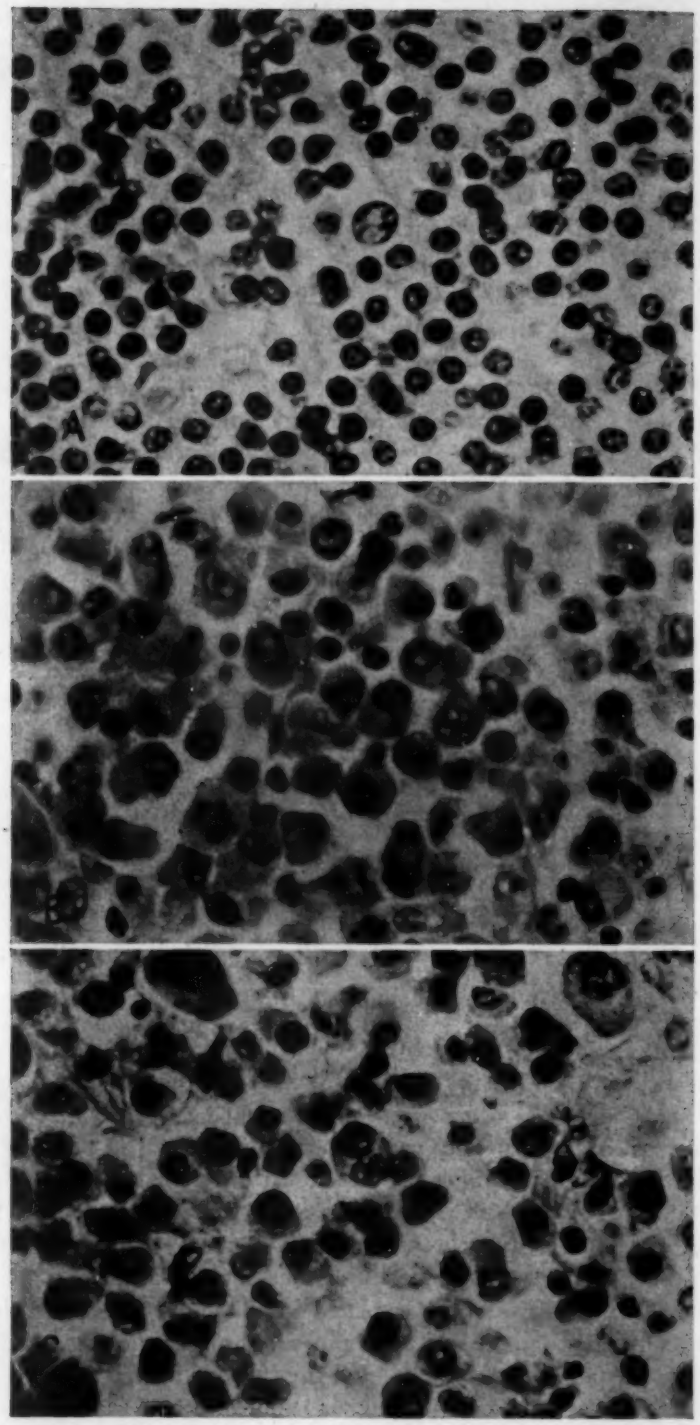

Fig. 9.—Photomicrographs illustrating the cytologic character of multiple myeloma. *A*, myeloma composed of predominantly small, uniform tumor cells that have a superficial resemblance to plasma cells. In the center of the field there is a tumor cell with a substantially larger nucleus. Myelomas showing this cytologic appearance are commonly designated as plasma cell myeloma or plasmacytoma. × 450.

B, another myeloma whose cytologic appearance is dominated by cells considerably larger than those resembling plasma cells. A number of the large tumor cells show mitotic figures. × 450.

C, still another myeloma composed predominantly of larger cells, including some with very large, hyperchromatic nuclei, but showing also an appreciable number of smaller cells, some of which likewise present hyperchromatic nuclei. × 450.

Among the tumors in our material there were a number which cytologically seemed to be intermediate between the predominantly small cell and large cell myelomas. These tumors resembled the small cell tumors in that they presented considerable cellular uniformity; however, their cells had somewhat larger nuclei on the whole, and there were an appreciable number of cells with very large nuclei and more than very occasional cells with two or more nuclei.

Correlation of the cytologic appearance of the myelomas in our material with the pertinent biochemical data, especially with the serum protein values (in those cases in which these values had been determined), yielded some interesting results (table). Specifically, we had 4 myelomas classed as large cell myelomas according to the criteria just

Correlation of Cytologic and Pertinent Biochemical Data

	Serum Albumin	Serum Globulin	Bence Jones Proteinuria
Large cell myelomas			
M. H.	2.8	7.6	Negative
A. R.	2.8	3.4	Positive
J. B.	2.0	14.0	Negative
M. K.	3.7	3.9	Negative
F. A.	...	...	Positive
G. P.	...	...	Positive
J. M.	...	...	Positive
M. S.	...	...	Negative
Intermediate group			
M. M.	3.3	6.9	Negative
P. L.	2.7	10.1	Positive
L. K.	...	...	Positive
M. K.	...	...	Negative
N. K.	...	...	Negative
Small cell myelomas			
E. O.	4.2	2.4	Negative
C. J.	4.8	1.9	Negative
N. G.	4.7	1.7	Negative
A. C.	4.1	2.5	Negative
S. B.	4.7	2.5	Positive

outlined and 2 that were intermediate in type, and with all of these the serum globulin values were significantly elevated, while the corresponding serum albumin values were diminished. On the other hand, there were 5 myelomas classed as small cell myelomas by the same criteria, and with all of these the serum albumin and globulin values were well within the normal range. As previously noted, Bence Jones proteinuria was observed with both cytologic types, though it was found more often with large cell myelomas. Hypercalcemia was observed about as frequently with one type as with the other.

It would appear from these data that the cases of large cell myeloma in particular are characterized by hyperglobulinemia. If this trend should be substantiated by further observations, we would have at least some insight into the puzzling question as to why hyperglobulinemia is observed in certain cases of multiple myeloma but not in others. Specifically, it would seem plausible that globulins may be produced or

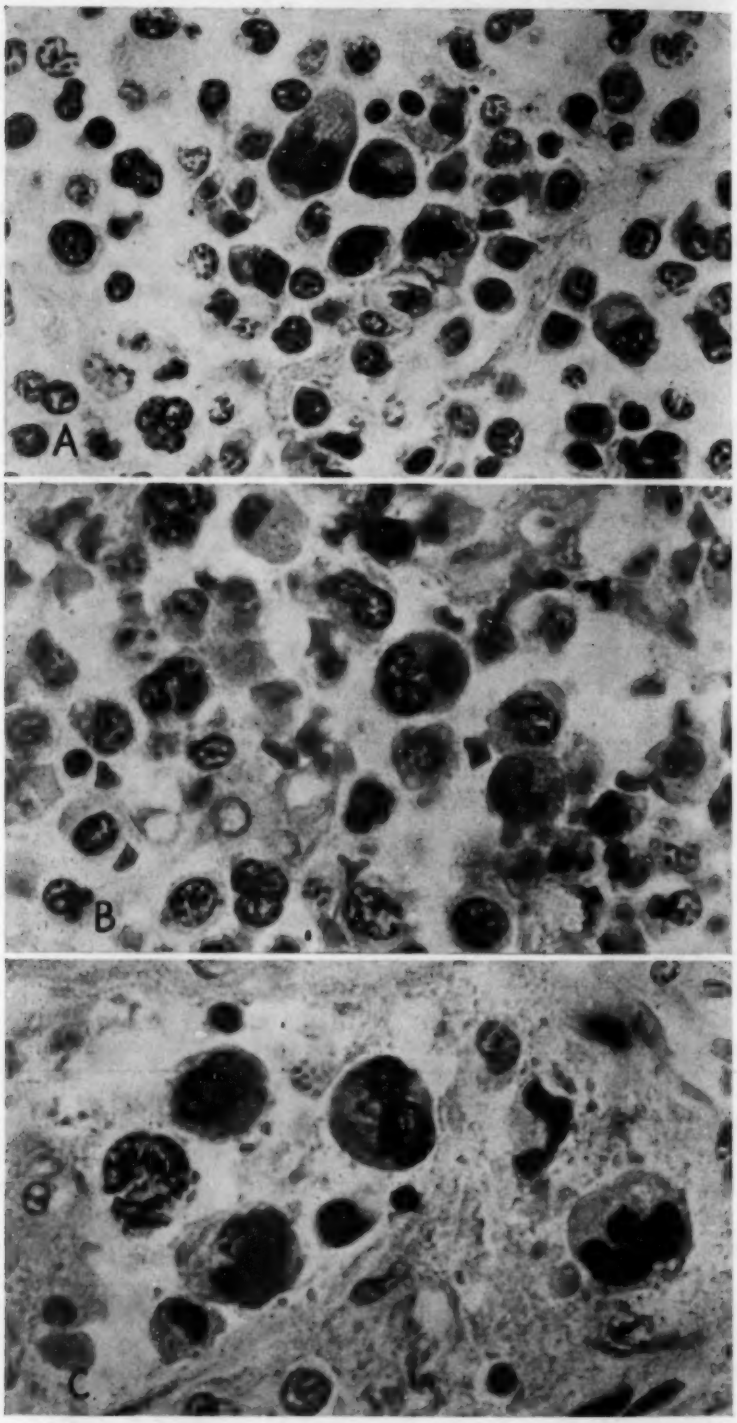

Fig. 10.—Photomicrographs illustrating the cytologic character of multiple myeloma (continued from fig. 9). *A*, a myeloma whose cytologic picture is dominated by comparatively large cells, though there is a sprinkling of smaller cells with nuclei not unlike those of the so-called plasma cells. A number of the cells have two or more nuclei, and some of these multinuclear tumor cells have markedly hyperchromatic nuclei. × 450.

B, another field of the same tumor, showing many cells with multiple nuclei. × 450.

C, another field of the tumor illustrated in *A* and *B*, showing a number of myeloma cells with bizarre hyperchromatic giant nuclei. × 450.

stored in appreciable quantity within the large and apparently less mature tumor cells and, by the same token, liberated and mobilized by way of the blood stream as these cells are broken down.

It is pertinent at this point to consider whether or not the large and small tumor cells respectively represent essentially different types of cells so far as their derivation is concerned. This question may not be of as great practical moment as it would be if, for instance, it had been established that the myelomas of relatively mature histologic appearance respond more favorably to treatment or necessarily pursue a more prolonged course than do the others; it is, however, germane to a better understanding of the nature of the neoplastic process. We are inclined to doubt whether there is any essential difference, save one of maturity, between the large and small tumor cells, since the predominantly large cell myelomas contain smaller cells in some places, to which it is possible to trace transitions, and the predominantly small cell myelomas contain occasional large cells (figs. 9 and 10). We would emphasize instead the unity of multiple myelomas and explain cytologic variations within them as expressions of their relative maturity or immaturity. This is the concept that has been advocated by Wallgren [84] in particular. One may draw a parallel with cancerous lymphomas, among which one observes some composed of relatively small, mature lymphocytic cells, others composed of somewhat larger, less mature lymphoblastic cells and still others composed of quite large, immature reticulum cells of more variable appearance, all of them derived from a common lymphoid stem cell (Mallory).

The identification of the common ancestral cell of multiple myeloma, however, is still a moot point. Some [85] have held that the tumor cells of multiple myeloma are abnormal hematic cells whose origin may be traced to the primitive reticulum cell of the bone marrow, and this theory has much to recommend it. Others have been impressed in certain cases by the alleged resemblance of the tumor cells to myeloblasts or myelocytes,[86] to lymphoblasts or lymphocytes, to erythroblasts,[87] to mature or immature marrow plasma cells,[88] to megakaryoblasts [89] or to hemocytoblasts.[90]

84. Wallgren, A.: Untersuchungen über die Myelomkrankheit, Upsala, Almquist & Wiksell, 1920; Baltimore, William Wood & Company, 1920.

85. Klemperer, P.: Beitr. z. path. Anat. u. z. allg. Path. **67**:492, 1920. Zadek, I.: Folia haemat. **58**:196, 1937.

86. Mieremet, C. W. G.: Virchows Arch. f. path. Anat. **219**:1, 1915. Symmers, D.: Ann. Surg. **67**:687, 1918.

87. Harbitz, F.: Norsk. mag. f. lægevidensk. **84**:212, 1923. Froboese.[25b]

88. Christian, H. A.: J. Exper. Med. **9**:325, 1907. Kracke, R. R.: Diseases of the Blood, ed. 2, Philadelphia, J. B. Lippincott Company, 1941, pp. 82 and 520.

89. Gunn, F. D., and Mahle, A. E.: Arch. Path. **26**:377, 1938.

90. Smith, R. P., and Silverberg, M.: Arch. Path. **21**:578, 1936.

Be that as it may, it should be emphasized that multiple myeloma presents a characteristic clinicoanatomic picture, centered around the skeletal manifestations of the disease and their sequelae, and that, with rare exceptions, this picture is readily distinguishable from that presented by any of the other neoplasms of hemopoietic derivation. So distinctive is multiple myeloma as a single and basically uniform disease complex that one is at a loss to understand why in some quarters it has been subclassified, presumably on the basis of cell type, into plasma cell, myeloid, erythroid and lymphoid myeloma. Indeed, there can be little doubt that multiple myeloma as discussed in this paper is a disease of unitary cell type, the variations in cytologic appearance reflecting stages in the maturation of the basic tumor cell. Specifically, regarding the histogenesis of multiple myeloma, we are inclined to hold with Wallgren,[84] Wood and Lucké,[91] Wintrobe [8] and others that this neoplasm consists of distinctive tumor cells which are probably of myeloid formative or hematic origin (though not clearly resembling any normal marrow cells or their immediate precursors) and are best designated noncommitally as myeloma cells.

Cognizance must also be taken of the idea stemming from Rustizky [92] and Lubarsch [93] that multiple myeloma represents a systematized disease of the hemopoietic apparatus and, as such, is not a true neoplasm but rather a hyperplasia related to the "leukemias." There can be no serious objection to holding that multiple myeloma is akin to chronic myelosis and cancerous lymphoma in the sense that it, too, belongs to the general family of neoplasms of hematic origin, although, as has already been indicated, it presents definite clinicoanatomic characteristics that sharply delimit it from these diseases. However, it is difficult to understand, even on theoretic grounds, how multiple myeloma can be regarded as anything but a cancerous neoplasm in the face of widespread formation of tumors within the skeleton, the tendency toward perforation of the cortices of affected bones and extension into the adjacent soft parts, the capacity of its cells to invade the blood stream and to metastasize to the viscera generally, in addition to involving the hemopoietic organs, and the consistent trend toward a fatal termination.

TREATMENT

Problems in therapy are concerned mainly with palliation, particularly the relief of distressing bone pain, general supportive measures and the handling of such complications as fractures and compression of the spinal cord. In regard to general supportive measures, the use of repeated transfusions to combat anemia when this is present and the avoidance

91. Wood, A. C., and Lucké, B.: Ann. Surg. **78**:14, 1923.
92. von Rustizky, J.: Deutsche Ztschr. f. Chir. **3**:162, 1873.
93. Lubarsch, O.: Virchows Arch. f. path. Anat. **184**:213, 1906.

of prolonged bed care should be emphasized. The consensus of radiotherapists seems to be that roentgen therapy, if judiciously employed, frequently, though not invariably, has value in palliation but that it has relatively little influence otherwise on the course of the disease except possibly when one is dealing with what appears to be a solitary myeloma.

Experience with radioactive phosphorus (P^{32}) in the treatment of multiple myeloma is still limited but is sufficient to indicate what may be expected at best and what its limitations are. In some patients, but by no means all, radiophosphorus therapy has resulted in appreciable subjective .clinical improvement, evidenced chiefly by relief of pain permitting restoration of more normal activity.[94] Apparently, no concomitant significant change in the roentgenographic appearance of the skeletal lesions has been observed. Reinhard and associates[95] concluded from their survey that radioactive phosphorus has not proved to be a really valuable therapeutic agent for the treatment of multiple myeloma and that the latter does not respond as favorably to that agent as it does to roentgen radiation. Indeed, it was felt that radiophosphorus therapy had shortened the life expectancy of 2 patients by producing severe leukopenia and thrombocytopenia.

The administration of "stilbamidine" (4, 4-diamidinostilbene) and "pentamidine" (4, 4'-[pentamethylenedioxy] dibenzamidine) in conjunction with a diet low in animal protein has recently been advocated by Snapper[96] for the treatment of patients with multiple myeloma, especially those with widespread but not large osteolytic lesions and with normally functioning kidneys. In such patients (15 had been so treated) he claimed to have observed a favorable influence on excruciating bone pain, but stated that the lesions persist and that treatment at best only checks the disease temporarily and does not cure it. Snapper pointed to the appearance of granules within the cytoplasm of the myeloma cells as an indication of the specific action of "stilbamidine" on these cells. The drug, however, has certain toxic effects, including injury of the trigeminal nerve in some cases, which is manifested in the development, following a delay, of facial anesthesia, and it seems that before employing the drugs in question for their palliative effect, one would be well advised to try first roentgen therapy in order to achieve the same result.

Aside from treatment of fractures, especially those of long bones, surgical intervention has a place in the relief of transverse myelitis resulting from extradural compression, which is accomplished by lami-

94. Low-Beer, B. V. A.; Lawrence, J. H., and Stone, R. S.: Radiology **39**: 573, 1942. Warren, S.: Am. J. M. Sc. **209**:701, 1945.

95. Reinhard, E. H.; Moore, C. V.; Bierbaum, O. S., and Moore, S.: J. Lab. & Clin. Med. **31**:107, 1946.

96. Snapper, I.: J. A. M. A. **133**:157, 1947.

nectomy. In regard to the latter, Jacox and Kahn [97] and Batts [4] have shown that this procedure, if followed by roentgen therapy, may permit complete recovery of function and even survival thereafter for a number of years. They emphasized that laminectomy should always be done before roentgen therapy is given, in order to prevent further damage being done to the cord by swelling subsequent to irradiation. Also, the question of ablation of a limb for myeloma sometimes arises, but only in connection with the comparatively rare, ostensibly solitary myeloma of a long bone. In cases of this type, as noted, one can never be certain that tumor is not present in other bones in spite of their negative roentgenographic appearance. It is pertinent, however, to cite the remarkable case reported by Stewart and Taylor [66] : The patient was alive and well eight years after forequarter amputation for a huge myeloma which had largely destroyed the upper third of the shaft of a humerus and was freely invading the muscles of the upper arm.

SUMMARY AND CONCLUSIONS

This study (based on 35 proved cases, in 18 of which autopsies were made) places emphasis on the clinical and anatomic features that characterize multiple myeloma. We conceive of the latter as a distinctive malignant disease of the skeleton primarily, which apparently takes its departure from the myeloid formative tissue proper. Anatomically, practically every bone may ultimately come to be involved more or less in a given case. The skeletal progress of the disease may be steady and rapid, sometimes from the beginning and sometimes after a static period. In some cases, also, before the disease becomes spread over the skeleton it may flourish in one bone (as a so-called solitary myeloma) for months or even years. Though at autopsy the skeleton may be found riddled through with foci of myeloma, it is only infrequently that gross foci are found in the viscera and other extraskeletal parts. Nevertheless, even in the absence of gross infiltrations, microscopic examination sometimes reveals smaller or larger numbers of myeloma cells within the spleen, the liver or lymph nodes and occasionally in other organs as well. Also, in some cases, myeloma cells may invade the blood stream. Ordinarily, under these circumstances, relatively few myeloma cells are found in the blood smears, but in an occasional case they may be so numerous as to create a leukemic blood picture (so-called plasma cell leukemia).

Although there are these points of resemblance to other neoplasms of hematic origin, it should be emphasized that multiple myeloma presents a characteristic clinicoanatomic picture, centered around the skeletal manifestations of the disease and their sequelae, and that with rare

97. Jacox, H. W., and Kahn, E. A.: Am. J. Roentgenol. **30**:201, 1933.

exceptions this picture is readily distinguishable from that presented by any of the other neoplasms of hemopoietic derivation. In this connection we have stressed the diagnostic significance of hypercalcemia, hyperglobulinemia (and its associated hematologic manifestations) and Bence Jones proteinuria, the not infrequent presence of atypical amyloidosis in association with myeloma and the well known cytologic renal changes of almost pathognomonic distinctiveness. The latter result commonly in more or less heavy albuminuria and often in renal insufficiency of a peculiar type. In regard to amyloidosis it was indicated that multiple myeloma is so often the basis for atypical amyloid deposits that the possibility of myeloma should be investigated in every case of idiopathic amyloidosis, even though the bones present no evidence of tumor either roentgenographically or on gross inspection at autopsy.

The tumor tissue in multiple myeloma tends characteristically to be composed of large aggregates of more or less compacted cells without any discernible intercellular material and without conspicuous supporting stroma. It has been recognized and must be emphasized that the cytologic picture is not the same in all specimens of multiple myeloma. Roughly, however, the tumors can be fitted into two general cytologic groups. On the one hand, there are those in which the tumor cells are quite uniform and predominantly small and have a superficial resemblance to plasma cells. It is to the myeloma showing this cytologic appearance that the name "plasma cell myeloma" or "plasmacytoma" is commonly applied. In the other group of myelomas the cytologic picture tends to be dominated by cells larger than those resembling plasma cells, but may be a rather variegated one. The dominant cell shows fairly abundant cytoplasm and has a large, round, oval or even reniform, pale stippled nucleus. In any particular tumor site examined, one may also find some of the smaller cells resembling plasma cells, or, on the other hand, find cells which are much larger than the dominant cells and frequently show nuclear atypism. Specifically, such atypical cells may present large and hyperchromatic nuclei, giant nuclei of bizarre shape, or two or more nuclei.

We are inclined to doubt whether there is any essential difference, save one of maturity, between the large and the small tumor cells. So distinctive is multiple myeloma as a single and basically uniform disease complex that one is at a loss to understand why in some quarters it has been subclassified, presumably on the basis of cell type, into plasma cell, myeloid, erythroid and lymphoid myeloma. Indeed, there seems to be little doubt that multiple myeloma as discussed in this paper is a disease of unitary cell type, the cytologic variations reflecting stages in the maturation of the basic tumor cell. Specifically, regarding the histogenesis of multiple myeloma, we are inclined to hold with Wallgren and others that this neoplasm consists of distinctive tumor cells which

are probably of myeloid formative or hematic origin (though not clearly resembling any normal marrow cells or their immediate precursors) and are best designated noncommitally as myeloma cells.

Correlation of the cytologic aspects of the myelomas in our material with the pertinent biochemical data strongly suggests that it is the large cell myelomas particularly which are characterized by hyperglobulinemia and, rather often, by Bence Jones proteinuria.

On the clinical side, in our cases of multiple myeloma, we found that the great majority of the patients were between 40 and 60 years of age, though some were in their 30's, and one was only 13 when the first manifestations of the disease appeared. Our data indicate that multiple myeloma may be slightly more prevalent in males than in females but do not support the often repeated statement that the condition is at least twice as frequent in males. In regard to the roentgenographic findings, we found that the picture conventionally held to distinguish multiple myeloma—that of many bones, including the calvarium, riddled by clearcut punched-out osteolytic defects—represents the exception rather than the rule and applies only to certain cases in which the disease is far advanced. Indeed, very often one observes merely some vaguely defined rarefactions in a number of the bones or a single exuberant tumor focus in some one bone (commonly a femur or a humerus, but sometimes a vertebral body, a rib or a clavicle, an innominate bone, a bone of the calvarium or some other bone) without obvious involvement of the skeleton generally. Sometimes (when myelomatous infiltration of the marrow is diffuse) skeletal changes may not be apparent at all roentgenographically, or the replacement of the marrow by tumor may be reflected merely by some osteoporosis. As for the calvarium, this not infrequently fails to show numerous punched-out rarefactions, even when roentgenograms show clearcut and widespread involvement of the rest of the skeleton. In such equivocal or initially obscure cases one must utilize fully all the available diagnostic cues to arrive at a combination of significant findings constituting probable or conclusive evidence of the presence of multiple myeloma. Marrow obtained by sternal puncture is often of great value in establishing the diagnosis.

Multiple myeloma has too variable a clinical course to permit of any dogmatic statement in regard to prognosis. It is true that the average length of survival after the onset of symptoms is not likely to be more than about two years. However, there are occasional patients with multiple myeloma, particularly those in whom the disease was apparently localized at the outset, whose course may be protracted over a number of years, sometimes as long as ten years or more. Problems in therapy are concerned mainly with palliation, particularly the relief of distressing bone pain, and general supportive measures, also the handling of such complications as fractures of bones and compression of the spinal cord.

MECHANISM OF LIPOPHAGE DEPOSITION IN ATHEROSCLEROSIS

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THERE are probably few diseases on which there have been more experimentation and speculation than atherosclerosis, and the result has been the accumulation of a vast and sometimes incoherent collection of facts, for which in many cases exactly opposed explanations have been given. For some years now I have been of the opinion that a simple and rational explanation is available for the main features of the disease and that the published work of Leary provides the last link in a chain of evidence that began with Thoma fifty or more years ago. In brief, Leary [1] suggested that when, through feeding, hypercholesteremia develops in rabbits the cells of the reticuloendothelial system take up the esterified cholesterol and after a latent period become detached, enter the blood stream, pass through the lungs to the systemic circulation and then, in the aorta, pass into the intima. That the reticuloendothelial system when stimulated by "colloid dyes and suspensoids" will deliver macrophages into the blood stream after a latent period was shown some time ago by Evans.[2] Leary [1] in his earlier papers suggested that entrance of the aortic intima was due to chemotaxis and that the site was chosen because of various stresses, but more recently [3] he has suggested that there is a certain sluggishness of the blood current in the aorta and the coronary arteries which causes the sticky macrophages to adhere to the intimal endothelium. Leary has, however, failed to pay full attention to a phenomenon that has been known for about a century and is a commonplace in all textbooks of pathology since Donders [4] and Gunning [5] (quoted by Thoma [6]) pointed it out, i. e., the fact that the corpuscular elements of the blood are in

From the Public Health Department.

1. Leary, T.: Arch. Path. **32**:507, 1941.

2. Evans, H. M.: Am. J. Physiol. **37**:243, 1915.

3. Leary, T.: Am. J. Path. **22**:634, 1946.

4. Donders, F. C.: Physiologie des Menschen, Leipzig, S. Hirzel, 1856, vol. 1; Nederl. Lancet, 1856, vol. 5; cited by Thoma.[6]

5. Gunning: Arch. f. d. holländ. Beitr. z. Nat.-u. Heilk, 1857, vol. 1; cited by Thoma.[6]

6. Thoma, R.: Text Book of General Pathology and Pathological Anatomy, London, Adam & Charles Black, 1896.

the central or axial stream, while the peripheral stream or *Randzone* is clear of cells, that, furthermore, the faster the blood stream is the wider is the clear *Randzone* (Sandison [7]) and that, as is well known, the mean velocity of the blood stream is higher in the aorta (300 mm. per second, according to Cowdry [8]) than in any other vessel. It also appears that Leary has no explanation to offer, except the vague term "chemotaxis," as to why the lipophage, and not the other white cells of the blood, forms the main feature of atheroma.

DIAPEDESIS

The problem to be faced is: Which influences bring the lipophages into the clear zone in contact with the endothelium, or, what amounts to the same thing, do away with the clear zone? First, it may be stated that studies on diapedesis since Schklarewsky [9] (quoted by Thoma [6]) and the more recent work of Clark and Clark [10] and Sandison [7] have shown that the most important factor in bringing the corpuscles into the peripheral zone is slowing of the blood stream and that the lighter the corpuscle the earlier it moves out. Schklarewsky, working under the direction of Recklinghausen and Helmholtz, demonstrated in glass tubes that in all fluids which hold in suspension small particles of unequal specific gravity, the heavier particles are collected near the axis of the stream, while the lighter particles circulate near the wall of the vessel. Furthermore, Schklarewsky proved that if the blood flowed in glass tubes which had contractions and dilatations in the course of their lumens, numerous white corpuscles accumulated in the dilated parts, in which the current was slower. It is further suggested that any small part of the arterial wall that projects into the axial stream will cause the peripheral stream at that site to cease to exist. A third factor that would destroy the differentiation between axial and peripheral zones would be any marked eddying.

Although mean blood velocity is at its highest in the aorta, a further analysis of the tendencies of the current in that vessel and in the large elastic arteries shows that the problem is not so simple. As Leary [3] pointed out, cardiac systole and diastole produce a certain intermittency of the stream. The aorta and the large elastic arteries act as a distensible reservoir that fills during systole and partially empties during diastole. This aortic reservoir is itself larger than the capacity

7. Sandison, J. C.: Anat. Rec. **54**:105, 1932.

8. Cowdry, E. V., in Arteriosclerosis, New York, The Macmillan Company, 1933.

9. Schklarewsky: Arch. f. d. ges. Physiol. **1**:602 and 657, 1868; cited by Thoma.[6]

10. Clark, E. R., and Clark, E. L.: Am. J. Anat. **57**:385, 1935.

of the left ventricle (Sainsbury [11]); so all its contents cannot leave it in each cardiac cycle—a portion must remain. In fact, some of the blood near the aortic cusps must reverse its direction when the valves are shut during diastole, and blood cannot escape out of the aorta into the common iliac arteries at a maximum rate, for then there would not be sufficient to insure an adequate supply to the more important common carotid arteries. Peripheral resistance in the vessels of the trunk and the limbs must be such as to insure retention of pressure in the aorta sufficient to provide enough blood for the brain despite the assumption of the upright posture by man. In other words, every particle of blood does not move in the aorta at the mean velocity characteristic of that vessel. This intermittency of some portions of the blood in the current gives an opportunity for the lighter corpuscular elements to enter the peripheral zone during diastole, while the heavier elements, owing to greater kinetic energy, have not the same tendency. These factors also apply to a lesser extent to the other elastic arteries. Widening of the vessel, the result of age or of disease, will further lessen the velocity and allow corpuscles to enter the marginal zone that otherwise would not have done so. The selection of the sites of atheroma will be discussed in further detail later.

The lipophage having entered the peripheral zone, it is necessary to suggest an explanation why it, and not the polymorphonuclear leukocyte, enters the lesion of atheroma, for the polymorphonuclear cells vastly outnumber the lipophages. Perhaps an indication is given by a consideration of which property of a corpuscle enables it to leave the axial current before another cell. That property is lightness. The lipophage, as it is packed full with fatty esters of a low specific gravity, must undoubtedly be lighter than the other leukocytes and so would tend to enter the peripheral zone at an earlier stage. Another factor to be considered would be its relative inertia as compared with the polymorphonuclear leukocyte. Leary [1] declared it to be ameboid, as it undoubtedly is, but it would be unnecessarily speculative to assume that this obese cell, stuffed so full of lipids that it resembles a honeycomb with barely distinguishable intervening strands of protoplasm, has the spontaneous activity of a polymorphonuclear leukocyte or other macrophage; it is probably far more subject to external influences, one of which would be the blood pressure. Should this cell, perhaps adherent to the endothelium, then pass a tentative pseudopod—why, one cannot speculate—into the intima, it would be subject to a difference of pressure on its surfaces, higher inside the blood stream, lower in the intima. As it is of fluid composition and relatively inert, its substance will tend to

11. Sainsbury, H.: The Heart as a Power Chamber, London, Frowde, Hodder & Stoughton, 1922.

flow from the higher pressure to the lower, i e., into the intima. It would of course be naive to assume that the pressure in the intima is zero while that in the blood stream is, say, 100 mm. of mercury; nevertheless it can be assumed that in the intima there is appreciably less pressure than in the lumen of the aorta. Proof of this is the demonstration by Winternitz and associates [12] that there are extensive capillary vasa vasorum in the aortic intima, originating from the lumen, particularly that of the older and diseased vessel. The pressure in these vasa must be less than that in the aortic lumen or blood would not flow into them, and the hydrostatic pressure in these vessels must be greater than that in the surrounding tissue or they would not function; therefore, a fortiori, the pressure in the intimal tissues must be less than that in the aorta. Winternitz and associates also demonstrated extensive hemorrhages originating from these vasa, in the intima, thus giving additional proof that the intimal pressure is less than the aortic. A similar conclusion must result from consideration of the dissecting aneurysm. However, the difference in pressure cannot be excessive, for then the aortic pressure would cause the intimal vasa to collapse. Blood pressure can thus be said to be a factor in the cause of atheroma, but not necessarily high blood pressure, merely the normal arterial pressure.

FATE OF THE LIPOPHAGE IN THE INTIMA

The lipophage having entered the intima, one finds the earliest sign of atheroma. As described by Leary, this is not even macroscopic, and one of the most contentious aspects of the disease has been the difficulty of determining which picture comes first, but Leary has extensive support, from as far back as Klotz and Manning [13] to as recent as Sjövall and Wihman,[14] whose material consisted of 1,380 cases, that the lipid is first of all intracellular. The lipophage, having entered the intima, would tend to move peripherally, no doubt helped by the same pressure influences, until it arrived at the internal elastic lamella. This structure, described by Dees [15] as a "feltwork" or "an exceedingly close-meshed plexus of very fine elastic fibres giving the appearance of a solid film-like sheet," has been described already by Aschoff [16] and Rosenthal [17] as a barrier to medial deposition of lipids of colloid form. How much more

12. Winternitz, M. C.; Thomas, R. M., and le Compte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938.

13. Klotz, O., and Manning, M. F.: J. Path. & Bact. **16**:211, 1911.

14. Sjövall, H., and Wihman, G.: Acta path. et microbiol. Scandinav., 1934, supp. 20, p. 1.

15. Dees, M. B.: Anat. Rec. **26**:161, 1923.

16. Aschoff, L.: Brit. M. J. **2**:1131, 1932.

17. Rosenthal, S. R.: Arch. Path. **18**:473 and 668, 1934.

would it be a barrier to the obese lipophage, whose natural egress from any situation is the lymphatic channel! In the aorta there are in fact 50 to 65 of these concentric barriers (Maximow and Bloom [18]). It is not suggested that the internal elastic lamella is an absolute obstacle, for it is fenestrated, and some cells make their way into the media, especially in those places where the elastica is less complete (Aschoff [16]), and can be found in the lymphatic channels of the adventitia (Leary [19]). Fox [20] stated that in the bird the internal elastic lamella is deficient and the lesion tends to extend more into the media. For the majority of these cells, however, imprisoned by the blood pressure on one side and the internal elastic lamella on the other, the intima is a mausoleum, and the further changes that occur therein need not be described in this paper.

WHY THE DISEASE AFFECTS MAINLY THE ELASTIC ARTERIES

If it is true that slowness of the blood is one of the main factors in the deposition of the lipophages, it must be clearly explained why the disease does not occur in other parts of the vascular system. First: Although the mean velocity of the blood gets less as the arteries become smaller, beyond the elastic arteries and especially in the veins the blood travels in a more even manner, less intermittently, than in the aorta and larger vessels; so the distinction of axial and peripheral currents tends to remain. In the veins the velocity, of course, increases. Second: Even if a lipophage should wander into the peripheral stream, it is required that the pressure be sufficiently high to push it out of the lumen. There is a fall of blood pressure from the aorta onward; the pressure, in fact becomes negative in the largest veins. Clark and Clark [10] described how a macrophage can even move from the tissues into the lumen of a capillary under the conditions of pressure existing therein. Thus the lower the pressure in a vessel the less the tendency for atheroma to occur. Third: Once the lipophage enters the intima it must be imprisoned therein by an internal elastic membrane. There are over fifty of these in the aorta, one in a muscular artery, and in an arteriole and a capillary none. In these small vessels the lipophage on leaving the lumen can easily find its way to a lymphatic channel except in tissues where there are none. If too many leave a capillary at any spot and the lymphatic vessels cannot cope with this, a xanthoma is the result. Such lesions have been caused in hypercholesteremic rabbits by trauma (Anitschkow [21]). If there are no lymphatic vessels, the

18. Maximow, A. A., and Bloom, W.: Text Book of Histology, Philadelphia, W. B. Saunders Company, 1943.

19. Leary, T.: Arch. Path. **21**:419, 1936.

20. Fox, H., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

21. Anitschkow, N.: München. med. Wchnschr. **60**:2555, 1913.

lipophages die eventually, leaving the cholesterol; this occurs in the cornea in arcus senilis, a feature not only of the elderly person but also of cholesterol-fed rabbits. In the veins the internal elastic lamella is far less well developed than in the arteries, and the vessel wall is also more vascular. These three features, i. e., more continuous flow, lower pressure and absence or incompleteness of elastic membranes, suffice to explain why atheroma is mainly a disease of the elastic arteries.

An attempt will now be made to show how the main pathologic observations and experimental results relating to atherosclerosis fit in with the hypothesis that has been developed.

LOSS OF ELASTICITY, DILATION AND LESSENED VELOCITY

Of all the facts relating to atherosclerosis the one on which there is most agreement is that with age the aorta tends to dilate as a result of loss of elasticity. Among the many authors who agree on this are: Adami,[22] Blumenthal and co-workers,[23] Duff,[24] Hueper,[25] Krafka,[26] Ophüls,[27] Plesch,[28] Rosenthal,[17] Thoma,[6] Wells,[29] Wilens,[30] Winternitz and associates.[12] This medial weakness is ignored only by the most enthusiastic cholesterol advocates and is the main argument in the armory of the anticholesterol protagonists, such as Duff.[24] Blumenthal and associates[23] have shown that it is accompanied by increased deposition of calcium in the media, as disclosed by microincineration, and Wilens[30] has demonstrated in postmortem material that this loss of elasticity is accompanied, where it occurs, by atheroma. If a vessel dilates, other things being equal, the velocity diminishes, for

$$V = \frac{A}{\pi r^2}$$

where V equals velocity in seconds and A equals volume per second. Using this formula and circumferences of aortas given by Kaufman and Aschoff, quoted by Krafka,[26] one finds that the average velocity of the blood of the aorta at 50 years of age is 60 per cent of that at 20 years of age. (This relation is illustrative only; it may not exactly

22. Adami, J. G.: Am. J. M. Sc. **138**:485, 1909.

23. Blumenthal, H. J.; Lansing, A. I., and Wheeler, P. A.: Am. J. Path. **20**:665, 1944.

24. Duff, G. L.: Arch. Path. **20**:81, 1935.

25. Hueper, W. C.: Arch. Path. **38**:162, 1944.

26. Krafka, J.: Arch. Path. **23**:1, 1937.

27. Ophüls, W., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

28. Plesch, J.: Lancet **1**:385, 1932.

29. Wells, H. G., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

30. Wilens, S. L.: Am. J. Path. **13**:811, 1937.

parallel the conditions in vivo.) Many years ago Thoma [6] suggested that a sluggish blood flow caused arteriosclerosis, without stating just how it happened, and he has been constantly criticized ever since. Adami,[22] for instance, stated that he had "never been able to grasp his explanation; furthermore I have never met anyone who has pretended to do so." Hueper [25] dismissed his suggestion that the lesions occur in situations where the velocity is lessened by pointing out that atherosclerosis is a disease of the aorta and not of vessels where the velocity is diminished. This point has, effectively, it is hoped, been dealt with earlier. There is no doubt that Thoma did not know why sluggish blood flow was so important, for the work on cholesterol feeding of rabbits had not been started and the lipophage theory had not been enunciated. Nevertheless, he took a great part in the description of the various phenomena connected with the leukocytes migrating from vessels and described Schklarewsky's work in which white corpuscles collected in the wider parts of glass tubes whose width varied. Thoma, furthermore, insisted that the particles of blood in actual contact with the wall of the vessel did not move at all. Not only is the general dilatation important, but local dilatations, i. e., aneurysms, are the site of atheroma. In experimental animals as well as in human subjects this is so—as, for instance, in the aneurysms of chicks described by Dauber and Katz,[31] in which atheroma occurred; in the fusiform dilations of the aorta described by Liebig,[32] and in the femoral artery of the side subjected to sympathectomy in the rabbit described by Harrison.[33] It should be remembered that atheroma is not usually confined to these places, but the incidence is greater there. The fact that in the adult the atherosclerosis is more marked in the abdominal aorta is to be correlated with the loss of elasticity (Wilens [30]) and the consequent greater relative widening there, this loss of elasticity perhaps being due to the extra head of pressure added to the normal pressure of the blood of the aorta on the assumption of the vertical posture. Be it noted that in the human infant the atheromatous streaks are mainly in the arch and the root of the aorta, and the older the subject the more the lesion spreads to the abdominal aorta (Ashoff [16]). Thus in the nonupright human being the site of atheroma is much the same as that in the rabbit, an effective answer to one of the main criticisms, that the rabbit lesion is not comparable with the human. Thus the theory that medial weakness and disease cause atheroma does not oppose the cholesterol theory; they are complementary to each other.

31. Dauber, D. V., and Katz, L. N.: Arch. Path. **36**:473, 1943.
32. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **159**:359, 1931.
33. Harrison, C. V.: J. Path. & Bact. **48**:253, 1939.

ATHEROMA AT THE SITE OF BRANCHING

In the rabbit and in man the lesion is well known to occur at the sites where arteries leave the aorta. In an artery that branches off at a right angle the atherosclerosis tends to surround the mouth of the vessel. When any particle changes its direction suddenly its momentum must slacken, and furthermore, eddies must occur here, both circumstances tending to bring the lipophage in contact with the endothelium. The lesion differs somewhat when the branch goes off at an oblique angle, for here the spur between the two vessels is the most affected. This would be expected, for when a branch goes off obliquely the axial and peripheral currents tend to remain separate, the lipophage remains in the axial stream, but the spur between the two vessels itself is in the axial stream. The striking and distortion of leukocytes against a spur of a dividing vessel are well described as observed in the living animal by Clark and Clark.[10]

OTHER ASPECTS OF BLOOD VELOCITY

As age advances, it does not appear that blood velocity lessens (Blumgart and Weiss [34]), although the incidence of atheroma increases. However, it is not the velocity of the blood of all the systemic and pulmonary vessels in which we are interested, but merely the velocity of that of the elastic arteries, which by virtue of their dilation, have a slower blood flow in old age. Presumably, the compensation that allows for a normal circulation time lies in the smaller vessels. High blood pressure is often associated with atherosclerosis, although it has been pointed out repeatedly that the disease regularly occurs with normal pressure. The blood velocity in fully compensated cases of high blood pressure is never increased; it is either normal or retarded (Blumgart and Weiss [35]). This is interesting, as the velocity of fluid in a tube, according to Poiseuille's Law, increases with increased difference of pressure between the ends of the tube. Thus in the body there must be compensations; probably an increased speed of flow in the smaller vessels plus the slower speed in the elastic arteries gives an average or slightly lengthened circulation time.

There is one condition which is constantly associated with changes in circulation time in man, and that is the basal metabolic rate. With an increased basal metabolic rate, i. e., in hyperthyroidism, the circulation time is diminished, and in hypothyroidism it is increased (Stewart and Evans [36]; Blumgart [37]; Tarr and co-workers [38]). This

34. Blumgart, H. L., and Weiss, S.: J. Clin. Investigation **4**:15, 1927.
35. Blumgart, H. L., and Weiss, S.: J. Clin. Investigation **4**:173, 1927.
36. Stewart, H. J., and Evans, W. F.: Am. Heart J. **23**:175, 1942.
37. Blumgart, H. L.: Medicine **10**:1, 1931.
38. Tarr, L.; Oppenheimer, B. S., and Sager, R. V.: Am. Heart J. **8**:766, 1933.

brings one immediately to the experimental work on cholesterol feeding of rabbits combined with manipulations of the thyroid apparatus, commenced by Murata and Kataoka [39] and followed up by others, such as Turner,[40] the gist of which is that the administration of thyroid diminishes experimental atherosclerosis and thyroidectomy increases it. The former would speed up the circulation, and the latter would diminish it. Unfortunately, the velocity of the blood is not the only factor involved in these experiments, for the blood cholesterol is changed as well, so the changes of circulation time are only part of the story. Turner and Khayat [41] have shown that administration of potassium iodide or of whole thyroid protects the experimental rabbit but that potassium iodide does not do so in the absence of the thyroid gland; thus potassium iodide does so by stimulating the gland. This leads up to the work of Page [42] on the di-iodide of ricinstearolic acid, a substance which prevents atheroma but increases blood cholesterol. If it can be shown that this substance stimulates the thyroid gland (the animals lost weight), it will be a pretty demonstration of the influence of blood velocity on atheroma with diminution of blood cholesterol excluded. However, the effect of iodine compounds on the thyroid gland is complex (Rosenthal [43]), and little can be assumed. The latest development, reported by Steiner and Kendall,[44] is of great interest, for by lowering the basal metabolic rate in dogs with thiouracil they could cause atherosclerosis after cholesterol feeding with regularity. This is one more nail in the coffin of the statement that experimental cholesterol-feeding arterial disease has no relation to human atheroma as it can be produced only in rabbits. From the human angle this demonstrates a danger of inducing coronary thrombosis in patients with thiouracil, one such case at least having already been reported in the literature (Himsworth and associates [45]). Another substance that increases the blood velocity is epinephrine, by action on the heart itself (Blumgart [27]). Anitschkow [46] described experiments in which cholesterol feeding of rabbits was combined with administration of epinephrine. The result was to prevent deposition of atheroma at the normal sites but to produce it at the sites of medial damage characteristic of the action of epinephrine.

39. Murata, M., and Kataoka, S.: Tr. Jap. path. soc. **8**:221, 1918.
40. Turner, K. B.: J. Exper. Med. **58**:115, 1933.
41. Turner, K. B., and Khayat, G. B.: J. Exper. Med. **58**:127, 1933.
42. Page, I. H.: Biol. Symposia **11**:43, 1945.
43. Rosenthal, S. R.: Arch. Path. **18**:827, 1934.
44. Steiner, A., and Kendall, F. E.: Arch. Path. **42**:433, 1946.
45. Himsworth, H. P.; Morgans, M. E., and Trotter, W. R.: Lancet **1**:241, 1947.
46. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933.

VASA VASORUM OF THE INTIMA

Winternitz and co-workers [12] ascribed atherosclerosis to hemorrhages and their sequelae, originating from extensive capillary networks in the intima. Vascularization of the diseased intima was described before by Robertson [47] and Paterson,[48] who denied that the vessels were present in the young healthy intima; even Winternitz and associates declared that in the latter circumstances vessels are sparse. Dauber and Katz [31] combined this theory with that of Leary, suggesting that the vasa vasorum become plugged with lipophages and so the intimal lesion is caused. Neither group of workers has considered the aorta of the young rabbit, in which the intima consists merely of an endothelial lining on the internal elastic lamella (Fox [20]). Nobody has described vasa vasorum of this site; if there were any, their presence would appear quite unnecessary; nevertheless, atheroma occurs there. This is not to deny the validity of the observations of Dauber and Katz. What they have described is the phenomenon of "leukocyte skimming" observed in the living animal by Sandison.[7] When the marginal stream is clear of leukocytes, capillaries leading off from the main vessel carry away only plasma, the process known as plasma skimming. Should the velocity in the main vessel have slackened to such an extent that leukocytes have passed into the marginal stream, the blood entering the capillaries carries with it leukocytes and plasma, and Sandison called the phenomenon leukocyte skimming. Dauber and Katz in their paper mentioned stagnation of the blood supply as a reason why the lipophage enters the capillary vasa, but they did not consider this lessened velocity (probably the most fundamental phenomenon in the causation of atherosclerosis) important enough to include it in their conclusion, and in a later review of the subject the same authors did not mention speed of circulation at all (Katz and Dauber [49]). These intimal vessels anastomose with adventitial ones, passing through the media. This gives an adequate explanation of lesions in the media featuring lipophages resulting from medial disease or experimental devices as described by Duff [24] and Ssolowjew.[50] Moon [51] formulated his anemia theory of atheroma, which he stated is the result of collapse of vasa vasorum caused by high aortic pressure. Winternitz and associates,[12] however, held that atheroma is the result of bleeding from extensive capillary networks, a precisely opposed conclusion. That the last-named authors entirely failed to consider the effects of pressure is shown by

47. Robertson, H. F.: Arch. Path. **8**:881, 1929.
48. Paterson, J. C.: Arch. Path. **22**:313, 1936
49. Katz, L. N., and Dauber, D. V.: J. Mt. Sinai Hosp. **12**:382, 1945.
50. Ssolowjew, A.: Ztschr. f. d. ges. exper. Med. **69**:94, 1929.
51. Moon, V. H.: Arch. Path. **3**:404, 1927.

their description of a sinusoid of the intima projecting into the lumen of the aorta like a hemorrhoid, which is of course impossible, as the pressure in an intimal vessel cannot be greater than that in the aorta.

BLOOD PRESSURE AND ATHEROSCLEROSIS

It has been stated by many authors, of whom Moschowitz [52] is one of the latest, that high blood pressure is the main cause of atherosclerosis, in spite of evidence to the contrary. They have cited the incidence of lesions in the pulmonary vessels in mitral disease, emphysema, etc., in the aorta proximal to the constriction in coarctation of the aorta, and in the aorta of the rabbit on the near side of an experimental narrowing, as well as the incidence of sclerosis of portal and mesenteric veins in hepatic cirrhosis, as proof of the high pressure theory. But the vessel proximal to a constriction always dilates if given time, so the velocity of its blood current is reduced, and this factor is consistently ignored, although it is present where high blood pressure cannot be, as in an aneurysmal dilation due to weakness of the wall of a vessel. The blood pressure in the pulmonary artery at its greatest, in mitral stenosis with heart failure, does not reach that in the normal aorta (Bloomfield and associates [53]); yet that artery can be extensively sclerosed. The pressure in a vessel must be high enough to provide a vis a tergo to push a lipophage into the intima, but need not exceed normal arterial pressures.

STRESS AND STRAIN

A bibliography of writers who have ascribed the localization of atheroma to sites of excessive stress and strain would run into some hundreds. However, the use of these terms is in reality a confession of ignorance and explains nothing. These terms are commonly used elsewhere in discussions of pathologic alterations of elastic tissue, such as hypertrophic emphysema of the lung, for which again a logical mechanical explanation can be given (Gordon [54]).

FIXATION AND MOBILITY

Although the sites of atherosclerosis are well known and noncontroversial, it is strange that two completely opposed views exist, one that the lesion occurs at sites of mobility (Harrison [55]), the other that it occurs at sites of fixation (Wilens [56]). There can be no questioning of

52. Moschowitz, E.: Am. J. M. Sc. **178**:244, 1929.
53. Bloomfield, R. A.; Lanson, H. D.; Cournand, A.; Breed, E. S., and Richards, D. W.: J. Clin. Investigation **25**:639, 1946.
54. Gordon, I.: Dis. of Chest **10**:180, 1944.
55. Harrison, C. V.: J. Path. & Bact. **36**:447, 1933.
56. Wilens, S. L.: Am. J. Path. **18**:63, 1942.

the experimental findings of these authors; it is their interpretations that must be at fault. Surely the early fatty streaks observed in the aorta are at sites of neither excessive fixation nor mobility. One might explain Harrison's findings in the terms of this paper by saying that intimal deposition occurred in the noncalcified parts of the aorta in his rabbits because the blood pressure in systole pushed those parts of the aorta out, caused relative widening of the vessel there and the lipophages entered the marginal zone of the slower stream. Atheroma occurred at the site of the silver cuffs of Wilens because in systole these projected into the axial stream. A similar explanation suffices for the deposition of atheroma where fractures occurred in the calcified media in Harrison's rabbits. The matter of eddies must also be considered but is difficult to interpret.

MACROMOLECULAR SUBSTANCES OF HUEPER

The interesting studies of Hueper [57] in which he injected at various times polyvinyl alcohol, pectin, acacia, methyl cellulose, sodium cellulose glycollate and hydroxyethylcellulose into experimental animals and caused atheromatous lesions in the aorta are well known. Hueper has preferred his own explanation of the intimal lesions, but to my mind his work fully confirms the theory of Leary. The reticuloendothelial cells of the liver and other organs become engorged with these substances, and in the case of methyl cellulose (Hueper [57a]) the foam cells are readily demonstrated in blood smears and are found in the aortic intima. Surely the most reasonable explanation of their presence in the latter is that they have come from the blood stream by the simple process of passing through the endothelium. It would be most interesting to know the specific gravity of the various substances Hueper used in the form in which they are present in the lipophages, in view of the hypothesis suggested earlier in this paper that relative lightness is the reason why these cells are the first to enter the marginal stream.

VALIDITY OF CHOLESTEROL FEEDING EXPERIMENTS

There has been a tendency of recent years to declare that the lesions caused in rabbits by feeding them on cholesterol has no relationship with human atherosclerosis. Leary [58] has already dealt with most of these criticisms, but to end this paper a few extra points are discussed. The statement that the experimental lesion is confined to the rabbit is now out of date, since Steiner and Kendall [44] can produce it in dogs. The different distribution in the rabbit's aorta as compared with the

57. Hueper, W. C.: (*a*) Am. J. Path. **18**:895, 1942; (*b*) **21**:1021, 1945; (*c*) Arch. Path. **39**:117, 1945; (*d*) **41**:130, 1946.

58. Leary, T.: Arch. Path. **21**:459, 1936.

human aorta is not a valid ground of criticism, for in the human infant, an animal that is not normally vertical, the site is much the same as in the rabbit. It is true that in the rabbits placed upright in jars by Wilens [59] the increased incidence of atheroma was not in the abdominal aorta, but the effect of posture in destroying the elasticity of the media of the lower part of the aorta is probably pronounced only after many years and not to be expected in the shorter experimental period, during most of which time the rabbits were asleep. That hypercholesteremia has no relation to the human disease can be upheld only by ignoring the work of Rabinowitch [60] on diabetes and, above all, by neglecting consideration of hereditary xanthomatosis (Bloom and co-workers [61]; Boas and Adlersberg [62]). In this disease high blood cholesterol is combined with extensive atherosclerosis and extraordinary susceptibility to coronary occlusion. Page [42] and Weinhouse [63] have rightly pointed out that human atherosclerosis is not always associated with hypercholesteremia, but in this connection it is necessary to stress a point in Leary's theory that Leary himself does not emphasize enough, i. e., that the lesion is not directly caused by the high blood cholesterol but by circulating lipophages, over years, perhaps only a few cells at any one time. Granted that the common cause of the circulation of these lipophages is high blood cholesterol, is it necessary to assume that this is invariably the case? Per contra, if there is hypercholesteremia, but for some reason the filling of the reticuloendothelial cells with lipids and their release from the liver are in abeyance, atheroma will be inhibited. This seems to be the most probable explanation of the fact that although alcohol has the effect of increasing experimental hypercholesteremia, atheroma is reduced, for in these rabbits deposition of lipid in the liver is also lessened (Eberhard [64]). Finally, it must be emphasized that the conceptions of the pure cholesterol school as enunciated by Leary and those of the school that insists on the primary influence of disease of the media (Duff [24]) are not or should not be opposed, the whole purport of this paper being that both are part of one and the same theory. I do not claim to have made any original observations but have picked the brains of many authors and out of a welter of facts produced, it is hoped, a coherent and novel synthesis. Probably the sole suggestion that has not been made by some one, somewhere, before, is that the lipophage, by virtue of its lightness, would be the first cell to come into contact with

59. Wilens, S. L.: Am. J. Path. **19**:293, 1943.

60. Rabinowitch, I. M.: Ann. Int. Med. **8**:1474, 1935.

61. Bloom, D.; Kaufman, S. R., and Stevens, R. A.: Arch. Dermat. & Syph. **45**:1, 1942.

62. Boas, E. P., and Adlersberg, D: J Mt. Sinai Hosp. **12**:84, 1945.

63. Weinhouse, S.: Arch. Path. **35**:438, 1943.

64. Eberhard, T. P.: Arch. Path. **21**:616, 1936.

the aortic endothelium and that, because of its relative inertness, it would be pushed into the intima by the blood pressure, and thus the main feature of atheroma would be formed.

SUMMARY AND CONCLUSIONS

The theory of Leary that atherosclerosis is caused by circulating lipophages is carried to its logical conclusion. It is pointed out that these cells, as all cells, would normally be in the center or axial current of the blood; that with slowing of the blood stream these cells would move into the peripheral or marginal stream and come into contact with the endothelium; that, because of their lightness, they would do so before any other cell, and then, because of their relative inertness, would be passively pushed into the intima by the blood pressure. Once inside the intima, the majority would be retained there by the internal elastic membranes which are impermeable to these fat cells. Atheroma is mainly a disease of the elastic arteries because, as they are filled only in systole and are partially emptied during the whole cardiac cycle, the current has a certain intermittency, which is less the case in other vessels that are farther from the heart. The disease also occurs in the elastic arteries because they dilate with age or with disease and thus the velocity of the blood in them is further reduced. The disease does not occur in other vessels because the current is less intermittent, the vis a tergo pushing the lipophage into the intima, i. e., the blood pressure, is less, and the internal elastic lamellas are either less well developed or do not exist. Much of the experimental work of the last few years is discussed in the light of this hypothesis.

DISTRIBUTION OF PARIETAL CELLS IN GASTRIC DISEASE

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BOSTON

THERE is now general agreement that the source of the hydrochloric acid of the stomach is the parietal cells of the gastric mucosa.[1] Although the determination of gastric acidity has become almost a routine procedure in the study of stomach disorders, particularly in cases of peptic ulcer and cancer, the state of the cells which secrete the acid has been left largely to conjecture.

An attempt was made to determine whether there are quantitative or qualitative differences in the parietal cells in conditions in which there is usually hyperacidity (peptic ulcer) as contrasted with conditions in which the acid is usually low or absent (gastric cancer). The problem is of more than academic interest, since Hurst [2] and others have reported that the absence of acid found so frequently with gastric carcinoma usually, if not always, precedes the onset of the malignant process.

HISTOLOGY OF NORMAL PARIETAL CELLS

The parietal cells appear first in an embryo of 12 weeks, arising from undifferentiated cells about the necks of the gastric glands. In the adult stomach the parietal cells of each gastric gland are distributed throughout the entire length of the gland but are more common in the upper third (fig. 1). Their name "parietal" is derived from the fact that they are situated on the outer aspect of the cell layers of the gland and have no obvious contact with the lumen (fig. 2). They are often called "acid" or "oxyntic" cells. The cytoplasm stains readily with any of the acid dyes, such as eosin or phloxine, and can be shown to have small canaliculi and minute granules, which are related supposedly to secretory activity. The cells are oval or wedge shaped and average about 20 microns in diameter. The cytoplasmic outlines are distinct. The nuclei are frequently multiple (as many as eight have been found in an individual cell), small, round or oval, and often hyperchromatic. Although mitoses have been reported to occur, I have never observed them.

From the Laboratory of Pathology, New England Deaconess Hospital.

This investigation was aided in part by a grant from the Anna Fuller Fund.

1. Babkin, B. P.: Secretory Mechanism of the Digestive Glands, New York, Paul B. Hoeber, Inc., 1944. Ivy, A. C.: Surgery **10**:861, 1941.

2. Hurst, A.: Lancet **2**:1023, 1929.

MATERIAL AND METHODS

Because at necropsy the stomach usually shows extensive autolysis, only specimens removed surgically were studied. Of the 200 examined, 81 had been resected for duodenal ulcer, 80 for gastric carcinoma and 39 for gastric ulcer. The specimens were placed in Zenker's solution soon after removal, often within an hour. Blocks were taken from the same representative areas in each specimen as far as possible and were designated by appropriate letters (fig. 3). Since relatively few of the 200

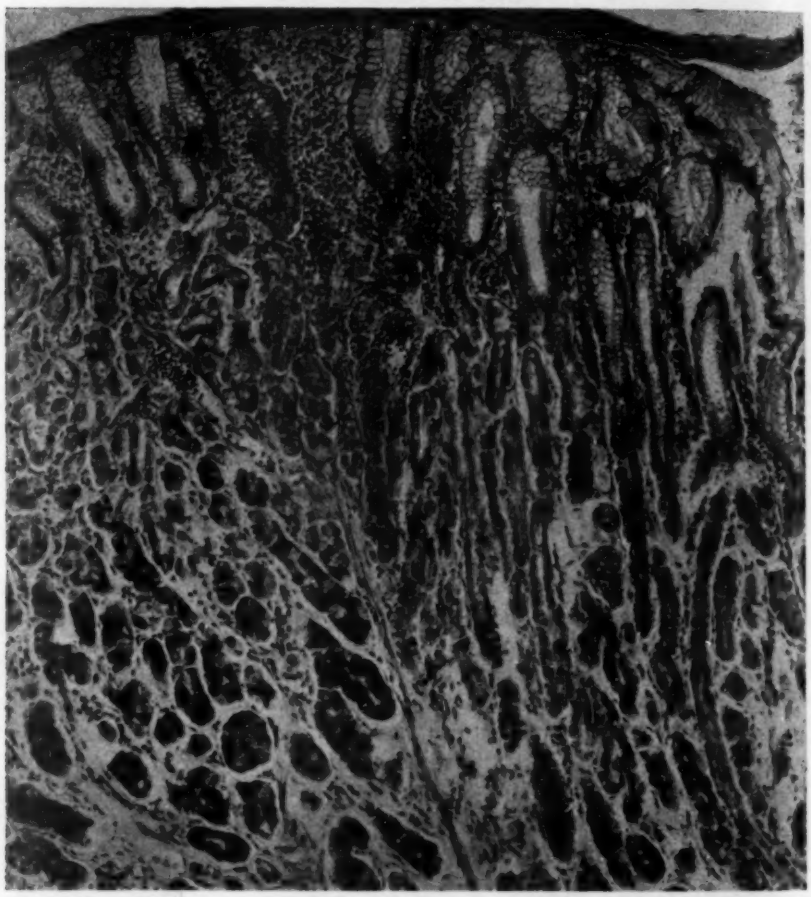

Fig. 1.—Abundance of parietal cells in a stomach resected because of duodenal ulcer. Eosin–methylene blue; × 100.

operations were total gastrectomies, the cardia of the stomach could not be adequately examined and is therefore not included in the study. All sections were stained with eosin–methylene blue.

An actual count of all the parietal cells in each of the blocks was too time consuming to be practicable. Therefore, a subjective estimate was made on each block as to whether the cells were abundant (fig. 1) or few (fig. 4). While there was a small amount of overlapping of the two groups, the great majority of micro-

scopic fields fell quite definitely into one or the other group. Such an estimate was made on each block.

In order to note qualitative changes in the parietal cells which might be seen in routine microscopic preparations, features such as size and shape of the cells, granularity or vacuolation of cytoplasm, density of nuclear material, size, shape and number of nuclei and distribution of cells in the gland were given particular attention.

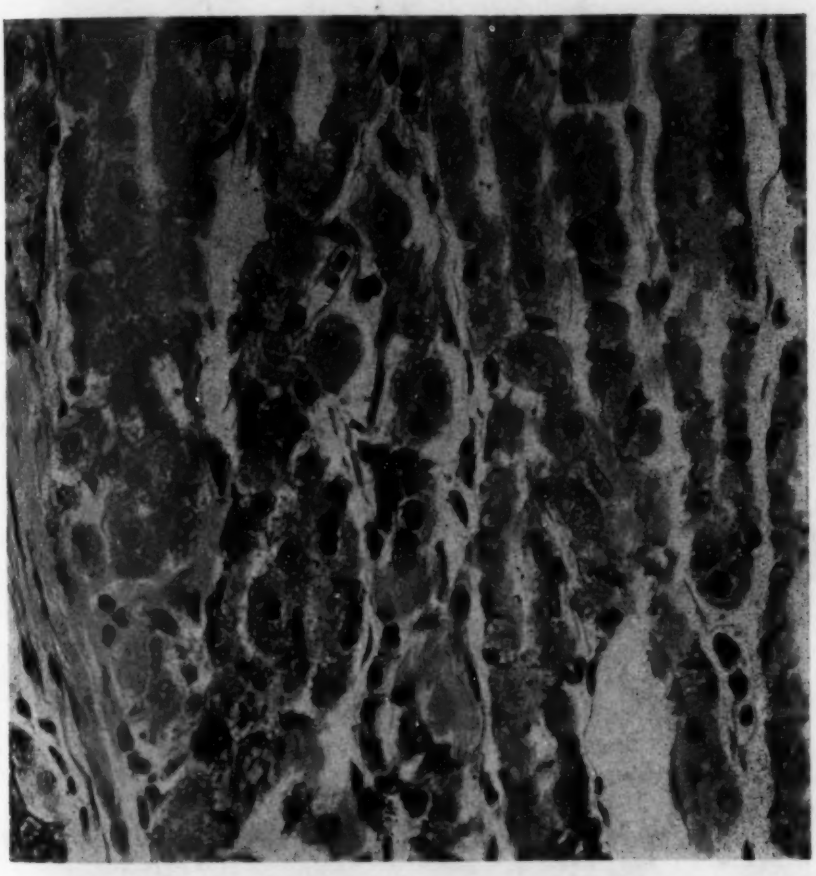

Fig. 2.—Higher power of a part of the section shown in figure 1. × 400.

RESULTS

The great majority of specimens were from patients 40 to 60 years of age. The ages of the patients with duodenal ulcer averaged 47 years; those of the patients with gastric ulcer, 51 years, and those of the patients with carcinoma, 55 years. Males predominated in a 5 to 1 ratio.

In all specimens the parietal cells diminished in number as the pylorus was approached. They were likewise somewhat fewer along

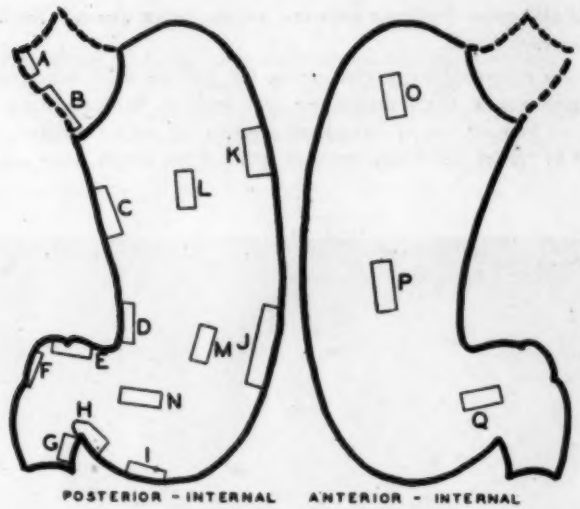

Fig. 3.—Chart showing areas from which blocks were taken for microscopic study.

Fig. 4.—Photomicrograph of a section showing few parietal cells. Eosin-methylene blue; × 100.

the entire lesser curvature as contrasted with opposite areas on the walls or greater curvature. This distribution corresponds in general with that found by Berger [3] in 8 normal stomachs in which he did actual counts of the parietal cells.

Quantitative Variations in Ulcer and Cancer.—It was found that the stomach could be divided roughly into four zones: the fundus, the body, the pyloric antrum and the pyloric canal. Except for the slight diminution of the number of parietal cells on the lesser curvature, any section of a zone fairly accurately represented the distribution of the parietal cells of that entire zone of any given specimen (fig. 5). To simplify the comparison of parietal cells in ulcer and cancer, only one block from each

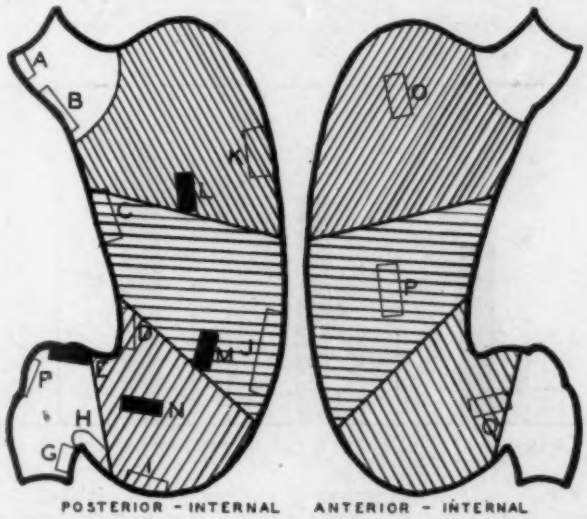

Fig. 5.—Chart showing the stomach divided into four zones for purposes of comparison.

zone needs to be considered. Sections E, N, M and L may be taken to represent the zones in which they lie, respectively.

The pyloric canal and the pyloric antrum showed few parietal cells in the great majority of cases of ulcer and of cancer (fig. 6). Furthermore, there was no significant difference in the percentage of cases showing this diminution between ulcer and cancer.

In regard to the body and the fundus, however, particularly in regard to the latter, there was a significant difference in the percentage of cases of ulcer and of cancer which showed few parietal cells; 54 per cent of the cases of cancer showed a small number of parietal cells

3. Berger, E. H.: Am. J. Anat. **54**:87, 1934.

in the fundus, while only 30 per cent of the cases of gastric ulcer and 10 per cent of the cases of duodenal ulcer showed such diminution.

There is, then, a tendency for cases of cancer to show fewer parietal cells than cases of ulcer, especially if the ulcer is in the duodenum. However, it can be readily seen from the graph (fig. 6) that a diminution of the number of parietal cells was not a constant finding in cases of cancer. Many stomachs with complete achlorhydria showed abundant parietal cells (fig. 7), and none of the cases of cancer showed complete absence of such cells.

Qualitative Changes.—As far as qualitative changes occurring in the individual parietal cell are concerned, as seen in routine stains there were no nuclear or cytoplasmic alterations which could be correlated with the presence or the absence of either peptic ulcer or gastric cancer. Cyto-

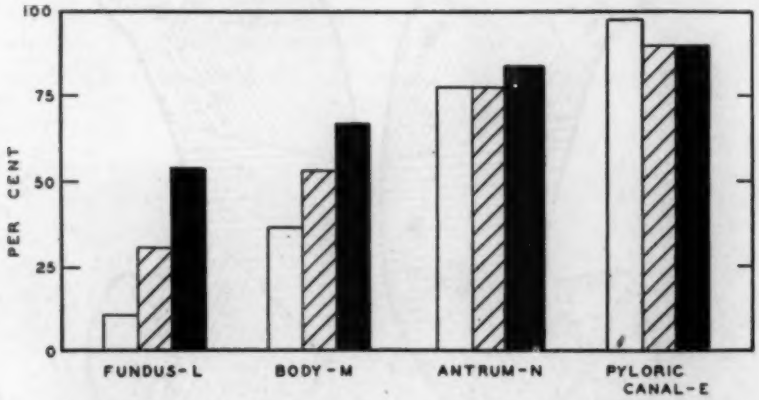

Fig. 6.—Graph showing percentage of sections with diminished parietal cells in cases of ulcer and of cancer. White columns represent duodenal ulcer; cross-hatched columns, gastric ulcer; black columns, carcinoma.

plasmic granules and vacuoles, possible precursors of the acid secretion, were frequently found associated with both types of gastric lesion.

COMMENT

According to Best and Taylor,[4] 60 per cent of cases of gastric carcinoma show complete absence of acid. It is somewhat surprising, then, that the acid-forming cells do not show fairly constant numerical or morphologic changes in cancer. Since the only change observed was a tendency for the parietal cells to be fewer and since abundant parietal cells were at times found with cancer and complete anacidity, one must look further for explanations of the problem.

4. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, ed. 4, Baltimore, Williams & Wilkins Company, 1945.

Numerous theories have been proposed to explain diminished gastric secretion in the presence of cancer. One of the more recent is that of Brunschwig,[5] who suggested that the cancer forms a "secretory depressant." Other explanations which have been offered include neutralization of gastric juice or suppression of secretion as a result of the action of

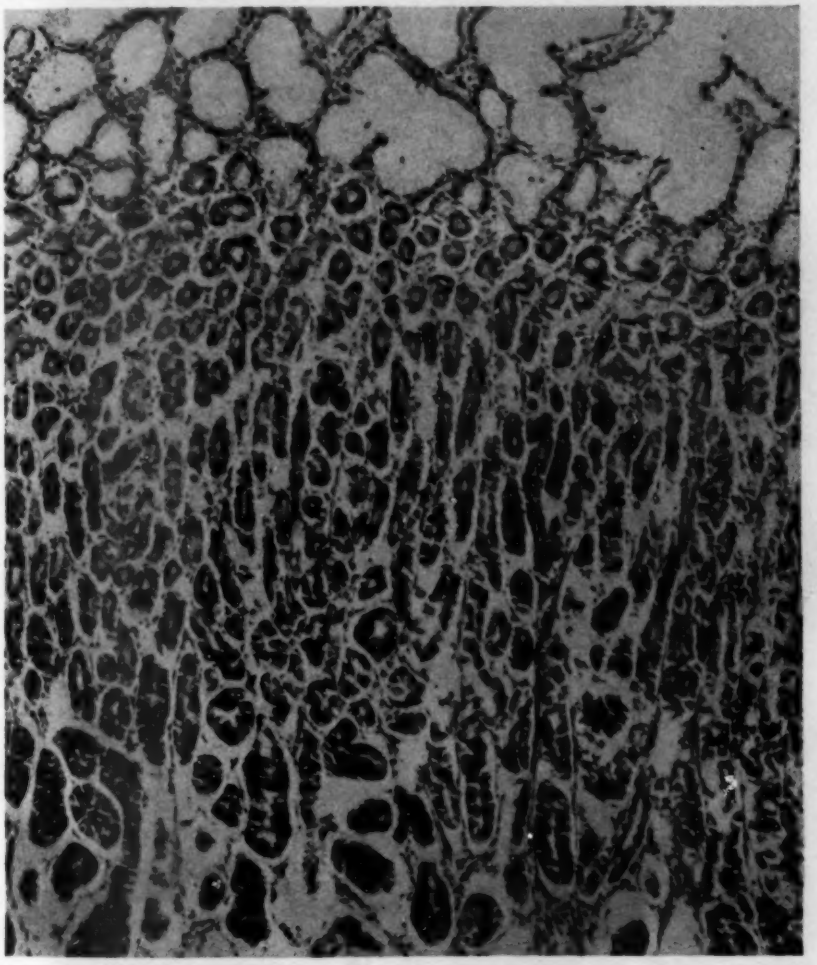

Fig. 7.—Abundant parietal cells in a stomach with cancer and with complete achlorhydria. Eosin-methylene blue; × 100.

the duodenal contents or mucus, interference with gastric nerves, chloride starvation and chronic gastritis. Polland and Bloomfield[6]

5. Brunschwig, A., and others: Surg., Gynec. & Obst. **70**:25, 1940.

6. Polland, W. S., and Bloomfield, A. L.: Bull. Johns Hopkins Hosp. **46**:307, 1930.

reviewed such explanations and concluded that "one can hardly evade the supposition that . . . gastritis may be the direct cause of the defective secretion."

This is a view held by numerous observers and one which is usually reached by a process of exclusion rather than by positive findings. It is true that it has been shown [7] that the incidence of chronic gastritis is higher in the fundus and the body of the stomach in cancer than it is in ulcer, and it is easy to assume that the diminution of the number of parietal cells which was found may be related to chronic gastritis. However, there is still left the difficulty of explaining anacidity in the presence of numerous parietal cells and in the absence of gastritis. Moreover, gastritis is a common condition and may be associated with hyperacidity as well as with diminished acid secretion.

It would seem, then, that there are probably several methods of bringing about the diminished secretion of acid which is seen in the presence of cancer of the stomach and that further investigation is warranted to determine more clearly the causes of this phenomenon and its relationship to the development of gastric cancer.

SUMMARY AND CONCLUSIONS

A series of 200 stomachs surgically resected for gastric carcinoma or for peptic ulcer of the stomach or the duodenum was examined with particular reference to qualitative and quantitative changes in the parietal cells in the different pathologic states.

The number of parietal cells diminishes as the pylorus is approached and is somewhat less along the lesser curvature than in corresponding areas on the walls or greater curvature.

The only quantitative change of significance was that in many cases of carcinoma there was a diminution of the number of parietal cells in the body and the fundus of the stomach, whereas in cases of peptic ulcer, especially cases of duodenal ulcer, such a diminution was less frequent.

A reduction of the number of parietal cells was not a constant finding in cases of cancer of the stomach; in many cases of cancer and complete anacidity there was an abundance of parietal cells. No stomach showed complete absence of such cells.

As seen in routine stains, there were no qualitative changes in individual parietal cells which could be correlated with ulcer or with cancer.

7. Meissner, W. A.: J. Nat. Cancer Inst. **5**:377, 1945.

SOME FACTORS INFLUENCING BROWN DEGENERATION OF THE ADRENAL GLAND IN THE SWISS ALBINO MOUSE

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THE TERM "brown degeneration of the adrenal gland of the mouse" has been used with reference to the pigment found mainly in the cells of the inner cortical zones, which appears brownish yellow in unstained sections as well as in those stained with hematoxylin and eosin. The pigmented cells usually form at the corticomedullary junction, but they may extend into the peripheral zones of the cortex, as well as among the cells of the medulla. Although brown degeneration of the adrenal gland of the mouse has been reported to occur under various conditions, its mode of origin is not well understood, for it is found mainly in certain strains of mice,[1] occurs most frequently in old animals,[2] is not correlated with the incidence of neoplasms in different strains of mice,[1a, b] and may be produced by administration of estrogen.[3]

Since the pigment of brown degeneration appears to be of a lipoid nature from its reactions with osmic acid and sudan stains (Cramer and Horning [2b]), the following experiments were carried out to determine whether qualitative and quantitative differences of the fat content of the diet would influence the incidence of brown degeneration in a strain of mice which normally has only a slight amount of this pigment in the adrenal glands when fed a commercial diet containing a relatively

From the Department of Anatomy, University of Rochester School of Medicine and Dentistry.

Part of the expense of this investigation was defrayed by the Fluid Research Funds of the University of Rochester School of Medicine and Dentistry.

1. (*a*) Kreyberg, L., and Ecker, R.: Avhand, utgitt av det Norsk. Vidensk. Akad. i. Oslo. I. Mat. Nat. Kl. **3**:1, 1939. (*b*) Blaisdell, J. S.; Gardner, W. U., and Strong, L. C.: Cancer Research **1**:283, 1941. (*c*) Dobrovolskaïa-Zavadskaïa, N., and Zéphiroff, P.: Compt. rend. Soc. de biol. **128**:971, 1938.

2. (*a*) Burrows, H.: J. Path. & Bact. **43**:121, 1936. (*b*) Cramer, W., and Horning, E. S.: ibid. **44**:633, 1937. (*c*) Blaisdell, Gardner and Strong.[1b]

3. (*a*) Lacassagne, A., and Raynaud, A.: Compt. rend. Soc. de biol. **124**:1183, 1937. (*b*) Burrows.[2a] (*c*) Cramer and Horning.[2b]

small quantity of fat. Some of the diets were also supplemented with tocopherols (vitamin E) to test whether the latter have an influence on the formation of this pigment. In view of the known changes of the adrenal glands of mice treated with estrogenic substances and the influence of these on fat storage in rats fed a diet rich in fat (Loeb [4]), the effect of supplementary injections of estrogen was also studied.

MATERIALS AND METHODS

Male and female mice of a homogeneous strain (Swiss albino) were used. The animals of the breeding colony were fed a commercial diet [5] and given tap water ad libitum. In most instances mothers and their young were started on the experimental diets on the day of delivery. In the groups given an estrogen, all members of each litter received subcutaneous injections of estradiol benzoate [6] in sesame oil (1 mg. per cubic centimeter) once weekly, doses of 0.01, 0.03 or 0.05 cc. being given for the first three weeks, and doses of 0.1 cc. during the subsequent weeks.

Approximately equal numbers of males and females of each age group were killed with illuminating gas and their adrenal glands fixed in Zenker's solution to which glacial acetic acid had been added to the concentration of 5 per cent. Paraffin sections, cut at 8 microns through the center of these glands, were stained with Verhoeff's carbolfuchsin for one hour at 65 C., destained with acid alcohol (3 per cent of hydrochloric acid in 80 per cent alcohol) for fifteen minutes and counterstained with Ehrlich's hematoxylin. Adjacent sections were stained with Ehrlich's hematoxylin and eosin; osmic acid, sudan IV, sudan black B, or nile blue sulfate for fats; by the Turnbull blue method for iron, or with Schultz cholesterol reagents.[7] The fluorescence of this pigment was observed in paraffin sections under ultraviolet rays. Adrenal glands (from selected animals) fixed in Levi's or Flemming's osmic acid were sectioned in paraffin and couterstained with Ehrlich's hematoxylin. Frozen sections of adrenal glands fixed in 10 per cent neutral solution of formaldehyde U. S. P. and cut at 15 microns were stained with osmic acid, sudan IV, nile blue sulfate, carbolfuchsin or Schultz cholesterol reagents. Other tissues from these experimental animals, prepared by these methods, will be described in a separate report.

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4. Loeb, H. G.: Proc. Soc. Exper. Biol. & Med. **51**:330, 1942.

5. The diet is marketed under the name "Wayne dog blox." See table 1 for composition.

6. The alpha-estradiol benzoate was supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

7. The following method was used to stain paraffin sections with sudan IV or sudan black B: Remove paraffin and hydrate sections to 70 per cent alcohol; cover sections with sudan IV (acetone-alcohol solution of Herxheimer) for two to five minutes or with sudan black B (a filtered saturated solution in equal parts of 70 per cent alcohol and glycerin) for thirty minutes; after use of either stain, wash in 70 per cent alcohol; counterstain if desired but avoid higher percentages of alcohol, which will remove the stain; hydrate, apply glycerin-gelatin (equal parts of glycerin and gelatin) and a cover glass. For the Schultz reaction sections are prepared as follows: Remove paraffin, hydrate and place sections in a 2.5 per cent aqueous solution of violet ferrous alum at 37 C. (or it can be used cold) for two to six hours, blot dry, add a few drops of equal parts glacial acetic and concentrated sulfuric acids to sections and apply a cover glass.

GENERAL STAINING REACTIONS OF THE PIGMENT OF BROWN DEGENERATION

Unstained Sections.—In sections of fresh or fixed (solution of formaldehyde U. S. P. or Zenker's) adrenal glands this pigment appeared brownish yellow and exhibited a similarly colored fluorescence under ultraviolet rays.

Stained Sections.—In frozen sections this pigment stained black with osmic acid, blue or light purple with nile blue sulfate, yellowish red with sudan IV, greenish blue with Schultz cholesterol reagents and bright red with carbolfuchsin. It usually stained much more intensely by these methods than did the lipids normally present in cortical cells.

In paraffin sections of adrenal glands fixed in Zenker's solution as described, this pigment stained yellowish brown with hematoxylin and eosin, yellowish red with sudan IV, black with sudan black, grayish black with osmic acid, blue or purple with nile blue sulfate, greenish blue with Schultz reagents and bright red with carbolfuchsin. Since cortical cells devoid of pigment did not stain with sudan IV or sudan black, osmic acid, nile blue sulfate or Schultz cholesterol reagents, this pigment, unlike the fat in the normal cortical cells, was insoluble in the usual dehydrating and clearing agents. After carbolfuchsin staining the pigment granules or globules were more distinctly depicted than after hematoxylin and eosin, osmic acid, sudan IV or sudan black staining. This pigment invariably gave a negative reaction for iron (Turnbull blue reaction). In tissue fixed in Levi's or Flemming's osmic acid solutions, the pigment stained black, the nonpigmented cortical cells were stained gray where the cells contained little lipoid material or black where lipoid material was abundant, and X zone cells undergoing degeneration were also stained black.

CLASSIFICATIONS OF THE STAGES OF BROWN DEGENERATION

The classification of the stages of brown degeneration proposed by Cramer and Horning,[8] subsequently used by Blaisdell, Gardner and Strong [1b] and others investigating this problem, was based primarily on studies of sections of the adrenal glands of relatively old animals stained with osmic acid, sudan III, or hematoxylin and eosin. This classification did not consider the pigment formed in the adrenal glands of younger animals where the X zone was present, or the condition of the cells during the progressive development of the pigment in different zones of the adrenal gland. Furthermore, the stains used did not depict the pigment as clearly as the carbolfuchsin (acid-fast) method used in this study.

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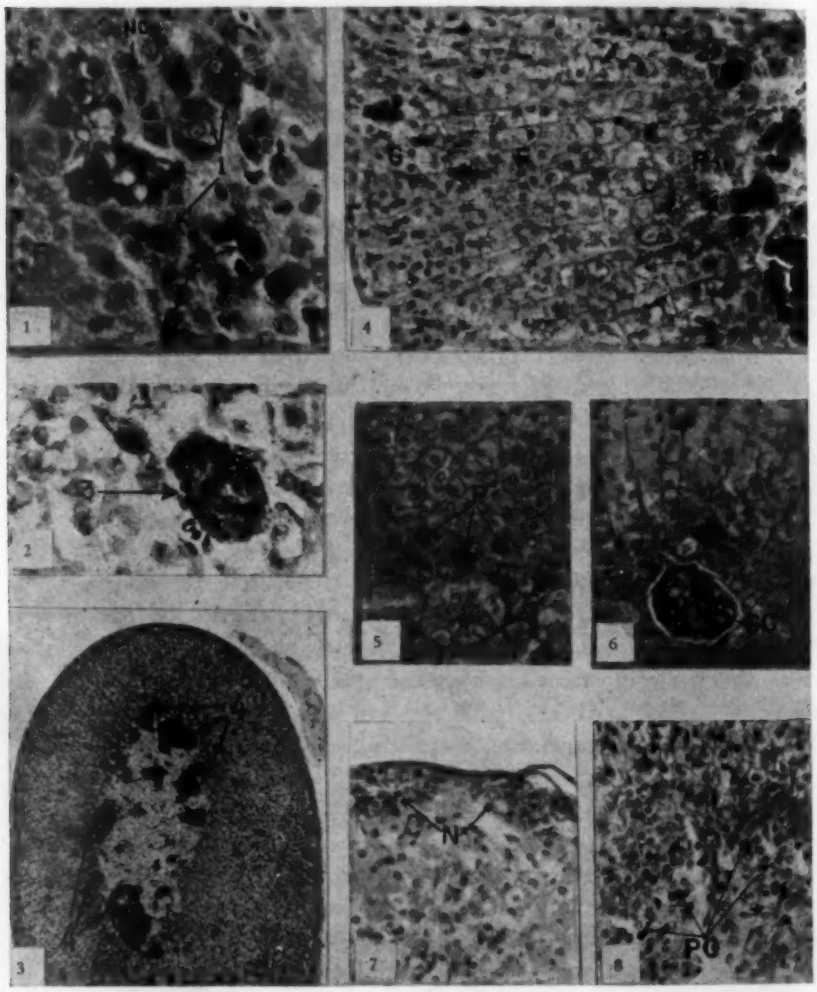
8. Cramer, W., and Horning, E. S.: Am. J. Cancer **37**:343, 1939.

The acid-fast and other staining reactions of the adrenal glands of young and old animals in our experimental and control series have led to the adoption of a classification of the stages of brown degeneration based on two major criteria: (1) the sequence and the relative extent



Figures 1 to 8

(See legend on opposite page)

of the pigment deposition within cortical cells, as well as the cytologic changes of these cells (qualitative), and (2) an estimate of the gross amount of this pigment in the whole adrenal gland based on representative sections of the gland (quantitative).

Qualitative Stages.—These stages are designated 0, 1, 2, 3 and 4. In stage 0 no pigment is present in any cells of the adrenal gland. In stage 1 granules of pigment appear in the cytoplasm of normal-appearing cells of the inner fascicular, the reticular or the X zone (fig. 1). In stage 2 pigment in the form of larger granules or globules, irregular in size, fills most of the cytoplasm in isolated cells of the reticular or the X zone, which have eccentrically located, pyknotic nuclei but intact cell membranes (fig. 1). In stage 3 multinucleated giant cells filled with pigment (fig. 2) are present in the reticular or the X zone, and the stage 1 and 2 cells appear in the glomerular and fascicular zones. In stage 4 a prominent ringlike area of pigmented cells forms at the corticomedullary junction, composed mainly of stage 2 and 3 cells. Cells of stage 1 were also found in other parts of the cortex, and if the X zone was absent, pigmented cells of various stages were found in the medulla (figs. 3 and 4).

Quantitative Estimate.—The total amount of pigment found in representative sections through the cortex and the medulla has been expressed as a graded series from no pigment (0) to the greatest amount of pigment observed (IV). This quantitative estimate appeared to be a necessary adjunct of the determination of the qualitative stages, since in any

EXPLANATION OF FIGURES 1 TO 8

Photomicrographs of paraffin sections of adrenal glands stained with carbolfuchsin (acid-fast) and Ehrlich's hematoxylin.

Fig. 1.—Inner fascicular (*F*) and reticular (*R*) zones of the adrenal cortex from a 217 day old male fed the high lard diet during the last one hundred days, showing normal-appearing cells (*NC*), stage 1 cells (*1*) containing granules or globules of acid-fast pigment but otherwise appearing normal, and stage 2 cells (*2*) with increased amounts of acid-fast pigment and pyknotic nuclei (× 196).

Fig. 2.—Reticular zone of the adrenal cortex from a 253 day old male fed the high lard diet for the last two hundred days. A stage 3 giant cell (*3*), containing several nuclei and large amounts of pigment, and several stage 1 cells with peripheral granules or globules of pigment are shown (× 196).

Fig. 3.—Adrenal gland from a 275 day old male fed the high lard diet for the last two hundred and fifty-four days. This shows a stage 4 reaction with a ringlike area of pigment (*4*) at the corticomedullary junction and various stages of pigment formation in other parts of the cortex and the medulla; the X zone had degenerated (× 19.5).

Fig. 4.—Zones of the cortex of the adrenal gland shown in figure 3. The pigmentation of cells of the glomerular (*G*), fascicular (*F*) and reticular (*R*) zones is shown (× 91.5).

Fig. 5.—Reticular zone of the cortex (above) and the medulla (below) of the adrenal gland of a male 200 days of age which was fed the stock diet, showing the usual amount of pigment (*P*) found in older animals (× 91.5).

Fig. 6.—Similar area from a 200 day old male showing the maximum amount of pigment found in adrenal glands of older animals fed the stock diet. One of three widely separated giant cells (*GC*) containing pigment is shown in this section at the corticomedullary junction (× 91.5).

Fig. 7.—Part of the adrenal cortex from a male fed the stock diet and given injections of an estrogen over a period of one hundred and fifty days. Note the nodular-like formations (*N*) containing pigment in the glomerular area (× 91.5).

Fig. 8.—Inner part of the adrenal cortex from a 10 day old male whose mother was fed the high lard diet during the last quarter of gestation and lactation. Pigmented cells (*PC*) are present in this area (× 91.5).

given qualitative stage the number of cells may not be the same in adrenal sections from different experimental groups and therefore alone may not be an index of the total pigment formation.

These two series of symbols representing the qualitative and the quantitative differences of pigmentation are used in table 2 to summarize the observations made on the adrenal glands in the various experimental groups to be discussed.

EFFECT OF DIETARY FAT, OF VITAMIN SUPPLEMENTS AND OF ESTROGEN ON BROWN DEGENERATION

The components of the diets used are shown in table 1. The low fat and the hydrogenated cottonseed oil ("crisco") diet were supplemented with vitamins A and D.[9]

Dietary Fat.—The adrenal glands of animals fed the stock diet, containing 4 per cent fat (iodine number: 86), had no pigment at thirty

TABLE 1.—*Diets*

Stock Diet,* per Cent		Experimental Diets		
		Low Fat,† per Cent	High Lard,† per Cent	"Crisco," per Cent
Protein.....22.0	Casein.....	20.0	20.0	20.0
Carbohydrate.....40.0	Starch.....	70.0	50.0	50.0
Salts..... 6.4	Salts.....	2.5	2.5	2.5
Fiber..... 5.0	Yeast.....	7.5	7.5	7.5
Moisture.....22.6	Lard.....	...	18.0	...
Fat..... 4.0	Cod liver oil.....	...	2.0	...
	"Crisco".....	...	...	20.0
	Vitamin A‡.....	20 U.S.P. units	...	20 U.S.P. units
	Vitamin D‡.....	2 U.S.P. units	...	2 U.S.P. units

* The stock diet consisted of "Wayne dog blox." The manufacturer's analysis is given here.
† The diet was deficient in tocopherols.
‡ Commercial concentrate of vitamins A and D was fed orally twice weekly.

days and relatively small amounts at the other age periods. With the exception of a few isolated pigment-containing multinucleated giant cells in the reticular zone of the glands of 4 animals over 200 days of age, stage 1 or 2 cells were the usual types observed (figs. 5 and 6). Mice fed the low fat diet, deficient in tocopherols and containing less than 1 per cent fat,[10] had no pigment in their adrenal glands at the various age periods. However, those fed the high lard diet, deficient in tocopherols but containing 18 per cent lard and 2 per cent cod liver oil (iodine numbers: 66 and 173, respectively), had considerably more

9. Vitamins A and D (concentrated), supplied by Distillation Products, Inc., Rochester, N. Y., were given orally twice a week as 20 U. S. P. units of A and 2 U. S. P. units of D.

10. C. G. Mackenzie, J. B. Mackenzie and E. V. McCollum (Biochem, J. **33**: 935, 1939) have shown that unextracted yeast and casein may contain up to 5.8 and 1.5 per cent lipid, respectively, and therefore this diet should be designated as low in fat rather than fat free.

pigment at each age period than did animals fed the stock diet. Diets comparable to the diet just mentioned or others containing a higher percentage of cod liver oil have been reported as causing pigment to be deposited in certain tissues of rats [11] which resembles in its staining reactions this pigment deposited in the adrenal glands of mice.

Evidence that pigmentation of adipose and other tissues of rats is due to the presence of unsaturated fatty acids in tocopherol-deficient diets [11e] and that an adequate supply of tocopherols prevents the pigmentation due to this dietary factor [12] suggested a study of the effects of such fats, with and without supplementary tocopherols, on the adrenal pigment of mice.

The highly unsaturated fraction of the fatty acids from cod liver oil (iodine number: 319), prepared according to the procedure followed by Dam and Granados,[11e] when fed at 3 per cent and 1 per cent levels in the low fat diet, replacing equivalent amounts of starch, produced extensive adrenal pigmentation. Retardation of growth was quite definite at the higher level but was only slight at the lower level of feeding.

In view of the observed influence of the dietary unsaturated fatty acids of tocopherol-deficient diets on adrenal pigmentation, a relatively saturated, hydrogenated vegetable oil ("crisco"—iodine number: 75) was substituted for the lard and the cod liver oil of the high lard diet. No pigment was observed in the adrenal glands of animals fed this diet or fed this diet supplemented with 1 per cent of the unsaturated fatty acid fractions of cod liver oil—so effective in causing pigmentation when incorporated in the low fat diet. That the protective action of the "crisco" diet was due to its tocopherol content [13] rather than to the reduction effected in unsaturation of the dietary fat is indicated by experiment in which tocopherols were given in conjunction with the high lard diet (see next section).

Tocopherols.—Animals fed the high lard diet had little pigment in their adrenal glands when given 10 to 15 mg. of tocopherols [14] orally once a week or when the tocopherols were incorporated in this diet to a concentration of 1 per cent. This dosage of tocopherols was more than adequate for normal reproduction of animals fed the high lard diet. Increasing the oral dose of tocopherols to 20 mg., given once a week, or the content in the diet to 3 per cent, resulted in complete absence of

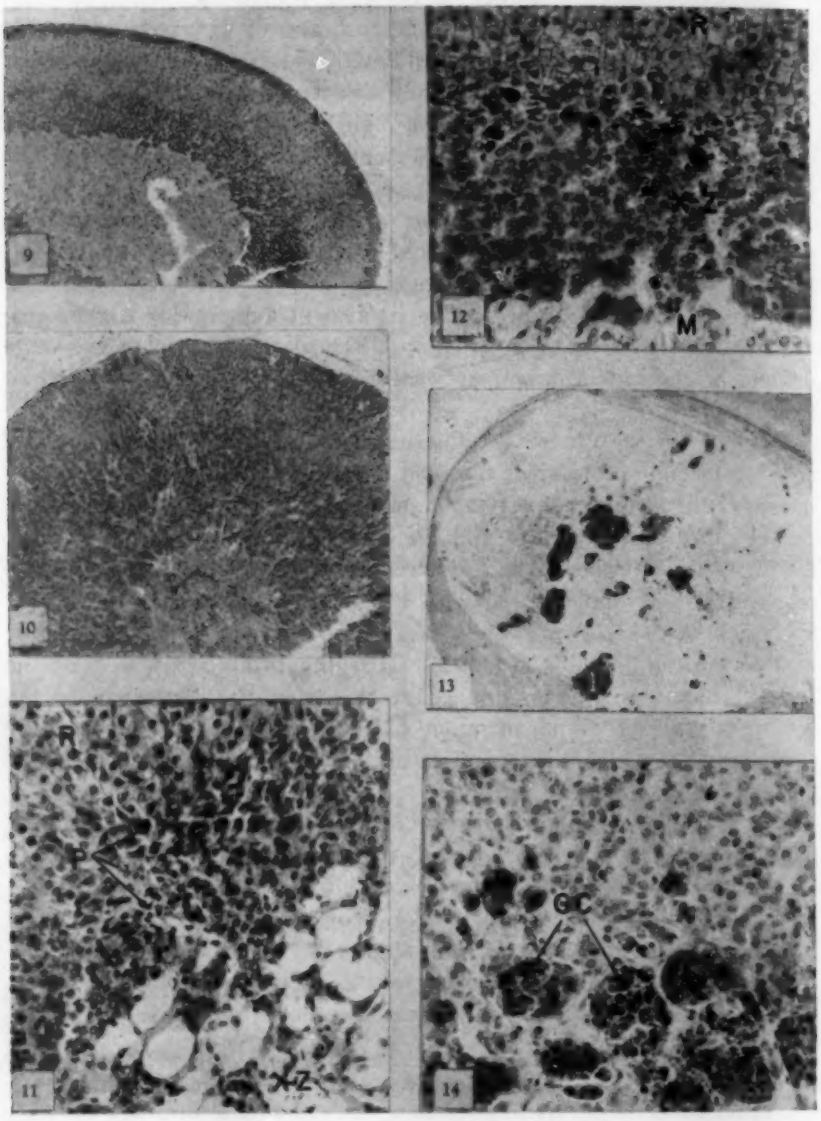
11. (*a*) Martin, A. J. P., and Moore, T.: J. Hyg. **39**:643, 1939. (*b*) Mason, K. E., and Emmel, A.: Yale J. Biol. & Med. **17**:189, 1944; (*c*) Anat. Rec. **92**:33, 1945. (*d*) Mason, K. E.; Dam, H., and Granados, H.: ibid. **94**:265, 1946. (*e*) Dam, H., and Granados, H.: Science **102**:327, 1945; (*f*) Acta physiol. Scandinav. **10**:162, 1945.

12. Mason and Emmel.[11b] Mason, Dam and Granados.[11d] Dam and Granados.[11f]

13. Hydrogenated cottonseed oil and other vegetable oils usually contain 1 to 2 mg. of tocopherol per gram of oil (P. L. Harris, personal communication).

14. The concentrated mixture of natural tocopherols (approximately 54 per cent tocopherols) was supplied by Distillation Products, Inc., Rochester, N. Y.

adrenal pigment. Four males and 4 females (data not included in table 2) fed the high lard diet for thirty days and then given the higher dose of tocopherols with this diet over a period of one hundred and twenty days had no greater pigmentation of their adrenal glands than mice fed



Figures 9 to 14

(See legend on opposite page)

this diet for only thirty days, indicating that deposition of pigment may be inhibited at any time by tocopherol feeding.

Animals fed the low fat diet containing 1 per cent fatty acids from cod liver oil and given 20 mg. of tocopherols orally once a week did have pigment in their adrenal glands. This may be attributed to the fact that such animals were receiving approximately 50 per cent more unsaturated fatty acids from cod liver oil than those animals fed the high lard diet.

Vitamins A and D.—These vitamins added to the low fat or to the "crisco" diet had no effect on adrenal pigment. The effect of their absence from the low fat diet was not studied. However, neither adrenal pigmentation nor any external sign of vitamin A deficiency was observed in 15 animals fed the "crisco" diet without supplemental vitamin A for one hundred and fifty days (data not included in table 2), although epithelial changes characteristic of this vitamin deficiency in the mouse [15] were observed in the vagina, the seminal vesicles, the testes and other organs. The epithelial metaplasia of the urogenital system appeared to be enhanced when animals restricted to this regimen were given injections of estrogen, causing early urinary obstruction and death. The effects of vitamin D deficiency were not studied.

Estrogen.—Since estrogen is known to produce brown degeneration in the adrenal glands, weekly subcutaneous injections of estradiol benzoate in sesame oil were given to animals fed the various diets. In mice fed on the high lard diet (with or without tocopherol supplementation)

EXPLANATION OF FIGURES 9 TO 14

Photomicrographs of paraffin sections of adrenal glands—all stained with carbolfuchsin (acid-fast) and Ehrlich's hematoxylin except for that shown in figure 13, which was stained with Schultz cholesterol reagents.

Fig. 9.—Part of the adrenal cortex and medulla from a 75 day old virgin female fed the high lard diet and given adequate tocopherol therapy orally twice a week. The therapy started at 30 days of age. Pigment is present in the inner reticular and the X zone. However, less pigment was present than was found in adrenal glands of animals fed this diet for a comparable period without tocopherol therapy (× 20.5).

Fig. 10.—Part of the adrenal cortex and medulla from a 62 day old female fed the high lard diet and given injections of an estrogen once a week. The X zone is well preserved. Note the diffuse distribution of pigmented cells throughout the cortex; pigment appearing to be in the medulla was in an intramedullary extension of a cord of X zone cells (× 20.5).

Fig. 11.—Part of the zona reticularis (*R*) and the degenerating X zone (*X-Z*) from a 125 day old female fed the high lard diet during the last fifty days. Pigment (*P*) is present in cells of the inner part of the reticular zone but not in the vacuolated, degenerating cells of the X zone (× 96).

Fig. 12.—Part of the reticular zone (*R*), the X zone (*X-Z*) and the medulla (*M*) from the adrenal gland shown in figure 9. Pigment is present in the reticular as well as in the X zone (× 96).

Fig. 13.—Section, adjacent to the one shown in figure 3, treated with Schultz cholesterol reagents. The areas of brown degeneration show a positive reaction with the Schultz reagents, whereas the normal lipid-containing cells are negative—the lipoid material of the latter was dissolved by the chemicals used in the paraffin-embedding technic (× 27.5).

Fig. 14.—Section through part of the corticomedullary junction from the adrenal gland of a male 217 days old which was fed the high lard diet during the last fifty days (× 96). The acid-fast pigment does not uniformly fill the cytoplasm of the giant cells (*GC*). When this diet was fed for longer periods, the giant cells contained more acid-fast pigment. Compare with the pigment in the giant cells shown in figures 2 and 3.

the injected estrogen exerted little or no effect on adrenal pigmentation. On the other hand, estrogen appeared to augment this process in mice fed the stock diet and to cause adrenal pigmentation, which would otherwise not have occurred, in those fed the low fat or the "crisco" diet. These injections resulted in persistence of the X zone in both sexes, whereas normally this zone was absent from the adrenal glands of males at 30 days of age and those of virgin females at 90 to 125 days of age in this strain. Nodules containing pigment were found in the glomerular zone of the adrenal gland after estrogen had been administered for one hundred days or more. (fig. 7).

Cholesterol.—In view of the positive Schultz cholesterol reaction found in areas of brown degeneration in paraffin (fig. 13) and frozen sections of adrenal glands, 1 per cent crystalline cholesterol was added

TABLE 2.—*Brown Degeneration in Adrenal Glands of Mice*

Basic Diet	Dietary Supplements and Treatment	Animals		Highest Qualitative Stage * and Estimated Quantity at Given Days of Age			
		Males	Females	30	50	100	>150
Stock diet	.....	31	15	0-0	2-I	2-I	3-I
	Estrogen.....	8	8	1-I	2-II	2-II	3-II
Low fat diet	.....	8	13	0-0	0-0	0-0	0-0
	Fatty acids, 3%.....	11	10	3-II	3-III		
	Fatty acids, 1%.....	6	6	2-II	3-III	4-III	
	Tocopherols,† fatty acids, 1%..	4	4	2-II	3-II		
	Estrogen.....	6	8	2-I	2-II	4-II	
High lard diet	.....	18	24	2-II	4-III	4-IV	4-IV
	Tocopherols ‡.....	6	6	1-I	2-I	2-I	
	Tocopherols §.....	6	4	0-0	0-0		
	Estrogen.....	10	6	1-II	3-III	4-IV	
"Crisco" diet	.....	14	13	0-0	0-0	0-0	0-0
	Fatty acids, 1%.....	4	4	0-0	0-0		
	Estrogen.....	9	4	2-I	2-I		

* The qualitative stage is indicated by the arabic, the quantitative estimate by the Roman numeral.

† Tocopherols were given orally once a week; dose, 20 mg.

‡ From 10 to 15 mg. of tocopherols was given orally once a week, or they were added to the diet to a concentration of 1 per cent.

§ Twenty milligrams of tocopherols was given orally once a week, or they were added to the diet to a concentration of 3 per cent.

to: the stock diet (pulverized), the high lard diet or the "crisco" diet (replacing equivalent percentages of lard and "crisco"). This cholesterol supplement increased but slightly the deposition of pigment in the adrenal glands when added to the stock diet (4 males and 5 females) for thirty to one hundred and fifty days, did not increase the pigmentation usually found with the high lard diet (10 males and 5 females) and produced no pigmentation of the adrenal glands when added to the "crisco" diet (10 males and 7 females). These data are not included in table 2. In all three groups the liver cells contained large deposits of fat which did not stain with carbolfuchsin in paraffin sections but did stain black with Levi's or Flemming's osmic acid fixation and red with sudan IV in frozen sections.

15. Wolfe, J. M., and Salter, H. P.: J. Nutrition 4:185, 1931.

COMMENT

From its staining reactions, the pigment of brown degeneration appeared to be of a lipoid nature. Yet the reaction to various fat stains (in paraffin sections) indicates that it, unlike the lipids in cells of the cortical parenchyma, was not soluble in the usual fat solvents. The reaction to nile blue sulfate suggests the presence of fatty acids.[16] However, this reaction, like that of osmic acid, sudan IV or sudan black, is not a specific histochemical test. The Schultz reaction usually indicates the presence of some form of cholesterol,[17] but it, too, should be accepted with caution, as indicated by Everett[18] from studies on the corpus luteum of the rat. If cholesterol constitutes a component of this pigment, it may be in the form of an ester or in combination with substances as yet unidentified.

The carbolfuchsin (acid-fast) staining reaction offers a better indication of the nature of the pigment, since this reaction is usually found in the tissues of animals fed tocopherol-deficient diets containing relatively high amounts of unsaturated fat. In vitro experiments of Endicott[19] likewise showed that the acid-fast reaction was found with unsaturated but not with saturated fats. Hass[20] has also suggested that this reaction may be due to polymerized peroxides of long chain fatty acids with several double bonds. The experiments reported here further verify the evidence that the acid-fast reaction of the tissues is correlated with the presence of unsaturated fats in the diet; for no adrenal pigment was found after a diet with a very low fat content or a diet with relatively saturated fat was fed. However, the pigment was found when diets containing unsaturated fat were fed or when fatty acids were added to the low fat diet. The qualitative and quantitative degrees of pigmentation were also related to the length of time the animals were fed such diets, for only small amounts of acid-fast pigment were found even in giant cells (fig. 14) after limited feeding of diets containing unsaturated fat.

Tocopherols are known to prevent deposition of acid-fast pigment in the tissues of rats fed tocopherol-deficient diets containing unsaturated fats. A sufficiently high dosage of tocopherols was likewise effective in preventing or limiting in the adrenal glands of mice the pigmentation due to the unsaturated fat contained in the diet. The inherent tocopherol content of the "crisco" diet, in addition to the relative sat-

16. Conn, H. J., and others: Biological Stains: A Handbook on the Nature and Uses of the Dyes Employed in the Biological Laboratory, ed. 2, Geneva, N. Y., The Commission on Standardization of Biological Stains, 1929, p. 75.

17. Whitehead, R.: J. Path. & Bact. **39**:443, 1934.

18. Everett, J. W.: Am. J. Anat. **77**:293, 1945.

19. Endicott, K. M.: Arch. Path. **37**:49, 1944.

20. Hass, G. M.: Arch. Path. **26**:956, 1183 and 1196, 1938; **27**:15, 1939; **28**:177, 1939.

uration of its fat component, also accounted for the absence of adrenal pigmentation in mice fed this diet with or without supplements of unsaturated fatty acids from cod liver oil.

A possible antagonism between tocopherols and estrogen was suggested by the clinical studies of Shute.[21] Such an antagonism would help to account for the presence of brown degeneration with prolonged estrogen treatment and the absence or inhibition of it with tocopherol therapy. However, when a preliminary test of this possibility was carried out with 8 spayed mice fed the stock diet and 2 mg. of tocopherols daily, all had vaginal cornification after being given a minimal dose of estradiol benzoate (0.20 micrograms) by subcutaneous injection, which would indicate that in the mouse such antagonism does not exist. This is in keeping with experiments by Halvorsen,[22] who also showed that tocopherols offered no counteraction to estrogen and progesterone treatment in the rabbit. Injected estrogen must, therefore, have altered the fat metabolism of the mice fed certain diets (stock, low fat and "crisco") to produce this pigmentation, for little or no pigment was found in the adrenal glands of animals fed these diets alone.

The type of cells that contain this pigment in the adrenal gland is not settled. Lacassagne and Reynaud[23a] suggested that the pigment found in the adrenal glands of mice after estrogen treatment was located in the reticuloendothelial cells. However, we observed this pigment in cortical parenchymal cells, also, which is in agreement with the reported capacity of such cells to phagocytose trypan blue.[23] Normally, the cortical cells considered to be the oldest (the inner reticular cells) usually are the first to contain this pigment. However, in advanced stages of brown degeneration, all areas of the cortex may be involved (fig. 4). The X zone cells appear to have the same ability to acquire this pigment as the cells of the outer cortical zones; for the adrenal glands of young whose mothers were fed the high lard diet during the last quarter of gestation and lactation contained pigment at 10 days of age, when most of the cortex consists of X-zone-like cells (fig. 8); moreover, before the X zone had degenerated normally or when it had been maintained by injections of estrogen (Vicari[24]), pigment was found within this zone (figs. 10 and 12). The X zone degenerated by vacuolation, as reported by others[25]; however, none of these degenerating cells con-

21. Shute, E.: Am. J. Obst. & Gynec. **44**:271, 1942; Urol. & Cutan. Rev. **47**:239, 1943.

22. Halvorsen, K.: Acta path. et microbiol. Scandinav. **21**:510, 1944.

23. Hett, J.: Ztsch. f. mikr. anat. Forsch. **7**:403, 1926. Cappell, D. F.: J. Path. & Bact. **32**:629, 1929.

24. Vicari, E. M.: Anat. Rec. **86**:523, 1943.

25. Howard-Miller, E.: Am. J. Anat. **40**:251, 1927. Deanesly, R.: Proc. Roy. Soc., London, s.B **103**:523, 1928. Whitehead, R.: J. Anat. **67**:387, 1933.

tained the pigment of brown degeneration (fig. 11). Pigmented cells were not found within the medulla as long as the X zone was present, but after the degeneration of the latter, pigmented individual or giant cells were found among the medullary cells. Once this pigment was formed in adrenal cells, they appeared unable to eliminate the pigment. It was also markedly resistant to postmortem autolysis.

From its staining reactions and its fluorescence under ultraviolet rays, the pigment of brown degeneration of the adrenal gland of the mouse appears to be similar to the ceroid of the dietary cirrhosis of the rat [26] or that found in the testes of mice treated with estrogens,[27] or the luteolipin of the corpus luteum of the rhesus monkey,[28] as well as the pigment found in the tissues of rats fed vitamin E–deficient diets with high contents of unsaturated fat.

SUMMARY

Brown degeneration was found in the adrenal glands earlier and in larger amounts after feeding diets with a high content of unsaturated fat to a strain of Swiss albino mice which has little of this pigment in the adrenal glands when fed a commercial stock diet with a low content of unsaturated fat, or no pigment when fed diets with very low fat or a relatively saturated fat content.

This pigment may be found in all zones of the adrenal cortex (including the X zone) as well as within the medulla in advanced stages of brown degeneration. No pigmented cells were found within the medulla if the X zone was present, but such cells were found in the medulla after the X zone had degenerated.

Injected estrogen appeared to increase slightly the formation of this pigment, whereas sufficient tocopherols (vitamin E) given in oral dosage or incorporated in the diet prevented or limited the formation of this pigment, provided the amount of unsaturated fatty acids in the diet was not excessive.

This pigment gave the Schultz cholesterol reaction in both frozen and paraffin sections. Cholesterol added to the diets in amounts to produce marked deposition of fat in the liver had little or no effect on the pigmentation of the adrenal cells.

From its staining reactions, this pigment appears to be similar to that found in the tissues of rats fed tocopherol-deficient diets with unsaturated fat content, the ceroid of the dietary cirrhosis of rats or that found in the testes of mice after prolonged estrogen treatment, and the luteolipin in the corpus luteum of the monkey.

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26. Endicott, K. M., and Lillie, R. D.: Am. J. Path. **20**:149, 1944.
27. Frantz, M.: Anat. Rec. **97**:388, 1947.
28. Rossman, I.: Contrib. Embryol. **30**:97, 1942.

HETEROTOPIC BLOOD FORMATION IN EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

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IN A previous publication [1] it was asserted that experimental cholesterol arteriosclerosis is a good means by which to elicit various potential reactions of tissues. In the course of experiments of this kind [2] it was found that tissue changes and vascular changes occurred in the brain and the skeletal muscle of rodents, sites where such changes had not been seen before. In the following pages an additional alteration is reported, which, too, appeared during experimental cholesterol arteriosclerosis in rabbits and guinea pigs, namely, heterotopic blood formation. It was seen frequently in the cortex of the adrenal gland, a few times in the spleen but only once in the kidney and in the lymph node. So far I have been unable to detect it in the liver.

In studies of experimental arteriosclerosis, Chuma,[3] Versé [4] and Schönheimer [5] observed hemopoiesis in the spleen; Versé [4] and Anitschkow [6] observed it also in lymph nodes. In spite of great attention given to microscopic changes present in the adrenal gland, no hemopoiesis has as yet been observed in this organ.

Jordan in his general review [7] of extramedullary blood formation did not mention secondary sites other than the liver, the spleen and lymph nodes. Bloom [8] reported hemopoiesis occurring in the rabbit's adrenal gland after ligation of the renal artery and vein.

According to Brannan,[9] heterotopic bone marrow has been found in the adrenal gland; it was always accompanied by adipose tissue and

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1. Altschul, R.: Arch. Path. **42**:277, 1946.
2. Altschul, R.: J. Neuropath. & Exper. Neurol. **5**:333, 1946; footnote 1.
3. Chuma, M.: Virchows Arch. f. path. Anat. **242**:275, 1923.
4. Versé, M.: Verhandl. d. deutsch. path. Gesellsch. **20**:67, 1925.
5. Schönheimer, R.: Virchows Arch. f. path. Anat. **249**:1, 1924.
6. Anitschkow, N., cited by Schönheimer.[5]
7. Jordan, H. E.: Physiol. Rev. **22**:375, 1942.
8. Bloom, W., in Maximow, A. A.,.and Bloom, W.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1942, p. 115.
9. Brannan, D.: Bull. Johns Hopkins Hosp. **41**:104, 1927.

was regarded as an embryonic defect. In anemic persons in whom heterotopic hemopoiesis has a compensatory function, no adipose tissue was present.

MATERIAL AND METHODS

Twenty-eight rabbits and 12 guinea pigs were used in cholesterol feeding experiments. These animals were fed milk, yolk powder and yolk cake; some rabbits received capsules with 0.3 Gm. of cholesterol. In the latter case, the animals were given milk and yolk, too, in order to facilitate the absorption of pure cholesterol. The animals died spontaneously or were killed when their death seemed imminent. The organs and tissues were fixed in formaldehyde solution or Susa fixative and embedded in paraffin. The sections were stained by various methods, but for the present investigation only the hematoxylin-eosin stain appears relevant.

RESULTS AND COMMENT

Blood formation was found in the adrenal cortex, one kidney and one lymph node of rabbits and in the adrenal cortex and the spleen of guinea pigs. It was seen in the adrenal cortex quite frequently, but only those cases (7 rabbits and 1 guinea pig) were considered in which megakaryocytes were present. For these animals the duration of the experiments varied from eighty-three to two hundred and thirty-four days. The developing blood cells were of the erythroblastic and myeloblastic series and were found sometimes in clusters but more frequently in rows or ribbons in the adrenal blood sinuses. The microscopic appearance was much in favor of intravascular hemopoiesis, originating from the endothelium. The hemopoiesis occurred either in the zona fasciculata or the zona reticulata of the adrenal cortex. A noteworthy fact was that the frequent marked alterations of the adrenal cortex did not prevent formation of blood in this organ.

In one kidney of a rabbit, young blood cells and megakaryocytes were observed between cortex and medulla, forming small clusters and ribbons in enlarged capillaries, the latter being surrounded by numerous foam cells.

As mentioned before, the spleen and one lymph node were also found to be hemopoietic, but since observations of hemopoiesis occurring in these organs have been reported by other authors, and since the spleen, the liver and lymph nodes often appear as secondary blood-forming organs, this fact will not be discussed further.

In the absence of megakaryocytes the formation of blood was not regarded as proved, even if the process was fairly obvious. It may well be assumed that in such cases continued search might finally have revealed some megakaryocytes, but as the number of "positive" cases was large enough to exclude an exceptional occurrence, unrelated to the experiment, the other cases were disregarded. It is quite probable that even other organs may be found ultimately to be the sites of heterotopic formation of blood now that attention has been called to this reaction.

The following questions arise:

1. Is the blood formation due to blood deficiencies, and has it nothing to do directly with the cholesterol feeding? Since the bone marrow suffers greatly in the course of experimental cholesterol feeding, the need for vicarious hemopoiesis appears quite plausible.

2. Is the heterotopic blood formation an embolic process?

3. Why is the adrenal cortex the site of blood formation rather than the liver and the spleen, which, by their embryonic development, are predestined to such an auxiliary function, as is seen in human pathologic conditions?

4. Is the heterotopic blood formation entirely a pathologic process, without a physiologic function?

To these questions, answers may be rendered as follows:

1. I was unable to find in the literature reports regarding leukopenias caused directly or indirectly by cholesterol feeding. Ignatowsky [10] observed, in his experiments with rabbits, a decrease in the number of erythrocytes and in the hemoglobin content. Okey and Greaves [11] observed anemia in guinea pigs which were on a cholesterol diet. Dubach and Hill [12] found macrocytic anemia. Vice versa, Kon,[13] and also Weinhouse and Hirsch,[14] found no changes in the blood of rabbits on a cholesterol diet, and Schwarz and Lichtenberg [15] reported a normal hemoglobin value. In my experience, which, however, does not extend to an examination of all animals used, blood counts and hemoglobin were normal. It also seems that the heterotopic hemopoiesis is too slight to compensate and hide any possible myelogenic anemia or leukopenia.

2. Heterotopic blood formation occurred in organs other than the adrenal cortex only in rare instances, so that an embolic displacement appears improbable. Moreover, the sinuses of the adrenal gland are wide enough to allow free passage of such cells and therefore would not retain them in clusters and ribbons.

3. The liver and the spleen are gravely affected in cholesterol arteriosclerosis. Much of the parenchyma of the liver is damaged, and most of the Kupffer cells are transformed into foam cells. In rabbits and, to a lesser degree by far, in guinea pigs the lymphatic tissue of the spleen is "outcrowded" by the immense number of foam cells and can well be called atrophic. Most of the elements of the reticuloendothelial system

10. Ignatowsky, A.: Virchows Arch. f. path. Anat. **198**:248, 1909.
11. Okey, R., and Greaves, V. D.: J. Biol. Chem. **129**:111, 1939.
12. Dubach, R., and Hill, R. M.: J. Biol. Chem. **165**:521, 1946.
13. Kon, cited by Chuma.[3]
14. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30**:856, 1940.
15. Schwarz, H., and Lichtenberg, H. H.: J. Biol. Chem. **121**:315, 1937.

are transformed into foam cells. Thus, in these two organs auxiliary blood formation may have been prevented by the pathologic process. However, one of the first organs to suffer in the course of cholesterol arteriosclerosis is the adrenal cortex. The cells of the parenchyma become enlarged as a result of an increase of their lipid content, and cholesterol crystals form in and between the cells. Moreover, necrotic areas with or without reparative inflammatory processes are seen. It seems, however, that only the parenchymal cells are grossly damaged; the lining cells of the sinuses show no distinct morphologic alterations. Thus heterotopic formation of blood from these cells may be possible. But it should be mentioned that Schönheimer [5] expressed the belief that in the course of cholesterol feeding the endothelial cells of the adrenal sinuses absorb fat.

4. One can hardly invoke in my cases an anemia with the necessity for an auxiliary formation of blood, especially since red and white cells are being formed heterotopically. It also appears that this formation of blood is not an embolic process. Moreover, it seems improbable that the damaged cortex would be chosen as the site for vicarious hemopoiesis (though this possibility should not be excluded). Therefore, one has to consider the possibility that the extramedullary blood formation observed in the adrenal gland and the kidney may be a pathologic process per se, and it might be classified as a regression of endothelium to an ontogenetically younger stage at which blood formation occurs. In this connection I wish to point out that a few years ago [16] I tried to demonstrate in the presence of human arteriosclerosis that endothelial cells had regressed to a younger, mesenchyma-like cell type. Thus, hemopoiesis occurring in the adrenal cortex and in the kidney in the course of cholesterol arteriosclerosis may well represent another incident of a regression of endothelial cells, for which one would have as the only factor suggesting an explanation the toxicity of excess cholesterol or its indirect action via damage of the liver.

It is noteworthy that other observers have not described hemopoiesis as occurring in the adrenal cortex during cholesterol feeding. As mentioned in the introduction, I was able to elicit changes in vessels of the brain and in skeletal muscle, although other experimenters have failed to do this, and I expressed the view that this discrepancy may be explained by the fact that I used heated (and thus probably oxidized) cholesterol. This same explanation may be applied to the present problem of heterotopic hemopoiesis. That is to say, the heterotopic formation of blood may be ascribed to the fact that in my experiments but not in those of the previous experimenters heat was used in baking the yolk cake, which increased the toxicity of the cholesterol. The regression or dedifferentiation of endothelial cells noted in both human and experi-

16. Altschul, R.: Arch. Path. **38**:305, 1944.

mental arteriosclerosis should not be considered as a proof that these processes are identical but only as an indication that under natural and experimental conditions the endothelium may react similarly.

SUMMARY

Extramedullary formation of blood occurs in association with experimental cholesterol arteriosclerosis. The question whether it constitutes an auxiliary function for aiding the damaged bone marrow is discussed. In the absence of anemia and leukopenia and because an embolic displacement can be excluded, the possibility is envisaged that the heterotopic hemopoiesis might be a pathologic process per se. In this case a regression of endothelium to the hemoblastic stage would be similar to the dedifferentiation of endothelial cells in human arteriosclerosis.

TOXICITY OF "STILBAMIDINE"

A Study of the Effects of Chronic Intoxication

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THE DIAMIDINES developed by York and others [1] have found many therapeutic applications.[2] They have been tried against trypanosomiasis, malaria, leishmaniasis and babesiasis, and as bacterial antiseptics. Their toxic action on tumor cells in vitro has been studied, and lately their action on multiple myeloma.

The pharmacologic and toxicologic studies of these agents have been incomplete. Moreover, some of the earlier studies have been complicated by the fact that infected animals were used.

Lourie and York [1] found that the maximum dose of "stilbamidine" (4,4-diamidinostilbene) tolerated is 50 mg. per kilogram by intraperitoneal injection in mice and 20 mg. per kilogram by intravenous injection in rabbits.

Similar results have been obtained by other investigators for "stilbamidine," "propamidine" (4,4′-[trimethylenedioxy]di-benzamidine) and "pentamidine" (4,4′-[pentamethylenedioxy]di-benzamidine).[3] Studies of acute and chronic effects have been conducted by several investigators.[4] The results have been variable but have indicated that the chief effects of the diamidines were on the liver and the kidneys.

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This work was made possible by a grant from the Council of Pharmacy and Chemistry of the American Medical Association.

"Stilbamidine" was supplied by Merck & Co., Inc.

1. Lourie, E. M., and York, W.: Ann. Trop. Med. **33**:289, 1939.

2. Fulton, J. D.: Ann. Trop. Med. **34**:53, 1940. Kirk, R., and Sati, M. H.: ibid. **34**:83, 1940. Adams, A. R. D., and York, W.: ibid. **34**:173, 1940. Kirk, R., and McDonald, D. R.: ibid. **34**:131, 1940. Daubney, R., and Hudson, J. R.: ibid. **35**:187, 1941. Snapper, I.: J. A. M. A. **133**:157, 1947; J. Mt. Sinai Hosp. **14**: 119, 1946.

3. (*a*) Way, E. L., and Chan, L. K.: J. Pharmacol. & Exper. Therap. **81**: 278, 1944. (*b*) Wien, R.; Freeman, W., and Schotcher, N. M.: Ann. Trop. Med. **37**:19, 1943. (*c*) Seager, L. D., and Rigdon, R. H.: Personal communication to the authors. (*d*) Broom, W. A.: J. Pharmacol. & Exper. Therap. **57**:81, 1936.

4. (*a*) Bischoff, F.; Sayhn, M., and Long, L.: J. Biol. Chem. **81**:325, 1929. (*b*) Devine, J.: Ann. Trop. Med. **32**:163, 1938; (*c*) **34**:67, 1940. (*d*) Daubney,

(Footnote continued on next page)

Our own experiments have been directed primarily to the study of the chronic toxic effect of "stilbamidine" as produced in rabbits and mice. "Stilbamidine" was administered to rabbits subcutaneously, while to mice it was given subcutaneously or by stomach tube.

MATERIAL AND METHODS

Rabbits and mice to which "stilbamidine" was given were killed at different periods after the administration of the drug. The following organs were fixed and studied histologically: heart, lung, liver, intestine, spleen, pancreas, kidney gonads and adrenal glands. No study was made of the nervous system.

The fixatives used were solution of formaldehyde U.S.P., Bouin's and Zenker's solution. Sections were stained with hematoxylin and eosin solution or with Pollack's trichrome stain. Sudan III was used for the study of fat in tissues and Best's carmine for the determination of the glycogen. We used 160 rabbits and 100 mice.

EXPERIMENTS ON RABBITS

The effects of doses of "stilbamidine" varying from 10 mg. up to 100 mg. per kilogram were studied; the dose was given in a single injection or was distributed in several injections. It was found that a single dose of "stilbamidine" of 75 mg. or more per kilogram kills a rabbit in the space of one or two days. An injection of 100 mg. per kilogram is generally lethal in the course of a few hours or a maximum of one or two days. If the amount of 100 mg. per kilogram is given not in one dose but in ten daily doses, the animal generally survives until a dose of 70 or 80 mg. per kilogram is reached. If a dose very close to the lethal dose (e. g., 50 or 60 mg. per kilogram) is given, the animal may not die in twenty-four or forty-eight hours. Generally speaking, it lives for one week. This delayed lethal effect has been investigated. During the course of treatment the rabbits lost weight, had some diarrhea, became weak and generally showed great congestion of the iris. Histologic sections of the iris showed large hemorrhages.

In order to determine the possible cause of the delay of the toxic effect in rabbits, several experiments were made. Since it is known that "stilbamidine" produced a fall in blood pressure, a suspension of epinephrine in oil, 0.02 to 0.013 mg. per kilogram, was given simultaneously with the "stilbamidine" and the treatment continued daily until the animal died. Epinephrine helped relieve the signs of toxicity in the period immediately following the injection but did not prevent the delayed effect of the drug. Experiments with ephedrine, 3 mg. per kilogram, gave the same result.

In order to counteract the loss of weight and of appetite, a solution of "amigen" and a solution of "amigen" and dextrose was given throughout some of the experiments, but no favorable results were observed.

Blood sugar and blood urea nitrogen were determined, and the van den Bergh test was made, in a group of 21 rabbits. Six were used as controls. To these a solution of sodium chloride was given once a week. The other 16 rabbits received

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R., and Hudson, J. R.: ibid. **35**:187, 1941. (*e*) Kirk, R., and Henry, A. J.: ibid. **38**:99, 1944. (*f*) Allen, J. W.; Burgess, F., and Cameron, C. R.: J. Path. & Bact. **56**:217, 1944. (*g*) Oasther, E. G., and Fidler, H. K.: Tr. Roy. Soc. Trop. Med. & Hyg. **39**:533, 1946. (*h*) Cameron, G. R., and Oakley, C. L.: J. Path. & Bact. **38**:17, 1934. Wien and others.[3b]

"stilbamidine," 20 mg. per kilogram, once a week. Blood sugar and urea nitrogen were determined once a week, on the day preceding the injection of "stilbamidine." The qualitative van den Bergh test was made near the end of the experiment. Blood sugar was determined according to the Folin-Wu method; urea nitrogen, by Keller's [5] micromethod.

The blood sugar showed a variable response. Often there was preliminary hypoglycemia, occurring in three to four weeks; this was often followed by hyperglycemia, and occasionally the hypoglycemia returned. Tables 1 and 2 show the variations of the blood sugar level as determined for the control rabbits and for the rabbits which received "stilbamidine."

TABLE 1.—*Blood Sugar Levels of Controls*

Rabbit	Mg. of Sugar per 100 Cc. of Blood Before Injection	Mg. of Sugar per 100 Cc. of Blood on Given Day After Injection of Saline Solution							
		7	21	28	35	42	49	56	63
170	98	85	115	95	105	102	115	120	...
171	105	97	100	100	100	98	115	120	...
172	95	95	96	99	109	106	107	107	...
173	95	97	95	98	107	106	112	105	...
181	117	116	102	126	104	...	...	...	...
183	112	127	106	132	122	120	...	...	...

TABLE 2.—*Blood Sugar Levels of Rabbits Treated with "Stilbamidine"*

Rabbit	Mg. of Sugar per 100 Cc. of Blood Before Injection	Mg. of Sugar per 100 Cc. of Blood on Given Day After Injection of "Stilbamidine"							
		7	21	28	35	42	49	56	63
174	92	92	112	108	Expired				
175	98	109	100	95	170	105	130	115	145
176	94	94	90	60	200	195	120	115	158
177	90	98	103	65	140	102	68	120	157
178	94	100	82	52	124	98	110	175?	140
179	98	109	117	85	120	138	145	154	152
184	128	121	111	124	108	Killed			
185	113	129	104	135	109	Killed			
186	?	...	...	...	134	102	66	121	140
187	117	120	105	140	Died				
189	?	120	102	?	Died				
190	?	...	...	...	142	144	139	136	...
191	120	...	...	...	74	202	116	204	95

The qualitative van den Bergh test was made on serums of both the controls and the experimental animals. While the control serums always gave negative reactions, the experimental serums always gave positive reactions after the fourth injection.

The blood urea nitrogen was determined for all these animals, but the fluctuations found both for the controls and for the experimental animals were so great that we cannot draw any conclusions.

Blood counts, including differential counts, were made for a few rabbits each of which received an injection of "stilbamidine" once a week for eight weeks. The red and white cell counts did not change significantly during the entire course of the experiment. The differential counts generally showed a shift to the left toward the end of the experiment and especially in the period immediately following the

5. Keller, A. G.: J. Lab. & Clin. Med. **17**:1146, 1931-1932.

injection of "stilbamidine." Stab cells and juvenile cells and occasionally a few myelocytes were observed. A few nucleated red cells, cells with vacuolated cytoplasma and microcytes were occasionally observed. All these probably reflected the toxicity of the drug.

Pathologic Observations.—Of all the organs examined, only the liver and the kidneys were found to be affected by the drug, and therefore our description is limited to these two organs.

(*a*) Liver: Grossly, the livers of many of the rabbits which received a large amount of "stilbamidine" presented a mottled appearance. Histologically, those of rabbits which had 10 mg. per kilogram of the drug showed some dilatation of the blood sinusoids and of the central vein; the hepatic cells looked normal, and there was no fatty change. Rabbits which received 25 mg. per kilogram were killed after six months, and their livers were found to be perfectly normal. The same observations were made in regard to rabbits receiving 40 mg. per kilogram given in four doses of 10 mg. per kilogram each. The livers of rabbits which had 50 mg. per kilogram and were killed after three days showed dilatation of the sinusoids and some infiltration around the portal tract. The hepatic cells appeared normal.

The livers of rabbits which received 75 mg. per kilogram in doses of 25 mg. per kilogram and were killed at the end of the third week showed increased fibrous tissue and had large, dense masses of histiocytes and fibroblasts around the periphery of the hepatic lobules. Among these cells, several small, newly formed bile ducts were present. Also present among the infiltrating cells were some characteristic cells which contained fine brownish-pigmented granules. These pigmented granules have been found only in the livers of animals which received "stilbamidine." An iron-free pigment, "an *Abnutzung* (wear and tear) pigment," was described by Lubarsch (1899), according to Mitsuda,[5a] in necrotic liver cells.

The fibrous infiltration of the livers of some animals was so extended as to occupy a great part of the lobule itself. The hepatic cells which came to lie in contact with the fibrous infiltrating cells had a homogeneous and hyaline appearance; they took eosin heavily. Centrally, these cells were delimited by other cells, which were enlarged and had rather empty cytoplasm. Sometimes this appearance was due not to presence of fat or glycogen but to hydropic degeneration of the cells. Most of the cells in this region were binucleated. The centers of the lobules were usually formed by healthy cells, although foci of necrosis were sometimes noted among the central hepatic cells (fig. 1).

Rabbits which received 100 mg. per kilogram in the course of ten days had great cellular infiltration of the liver around the portal canal, predominantly of fibroblasts or histiocytes with a few lymphocytes. There was also proliferation of small bile ducts and fibrous tissue at the periphery of the lobules. The cells which were near the infiltrating cells showed the same characteristic changes as those in the preceding group.

In the livers of rabbits which had 75, 80 or 100 mg. per kilogram in only one injection and died within a few days, no infiltration or fibrosis was ever observed, and no necrosis was present. Only a slight fatty metamorphosis was observed. The only typical feature was dilatation of the blood vessels. There were only 2 rabbits which survived a dose of 100 mg. per kilogram given in one injection. They were killed and autopsies made at the end of seven months. In one of them an entire lobule of the liver was destroyed, and some hemorrhages were present. Around the periphery of the lobules there was a large infiltration of histiocytes, with some

5a. Mitsuda: Virchows Arch. f. path. Anat. **248**:91, 1924.

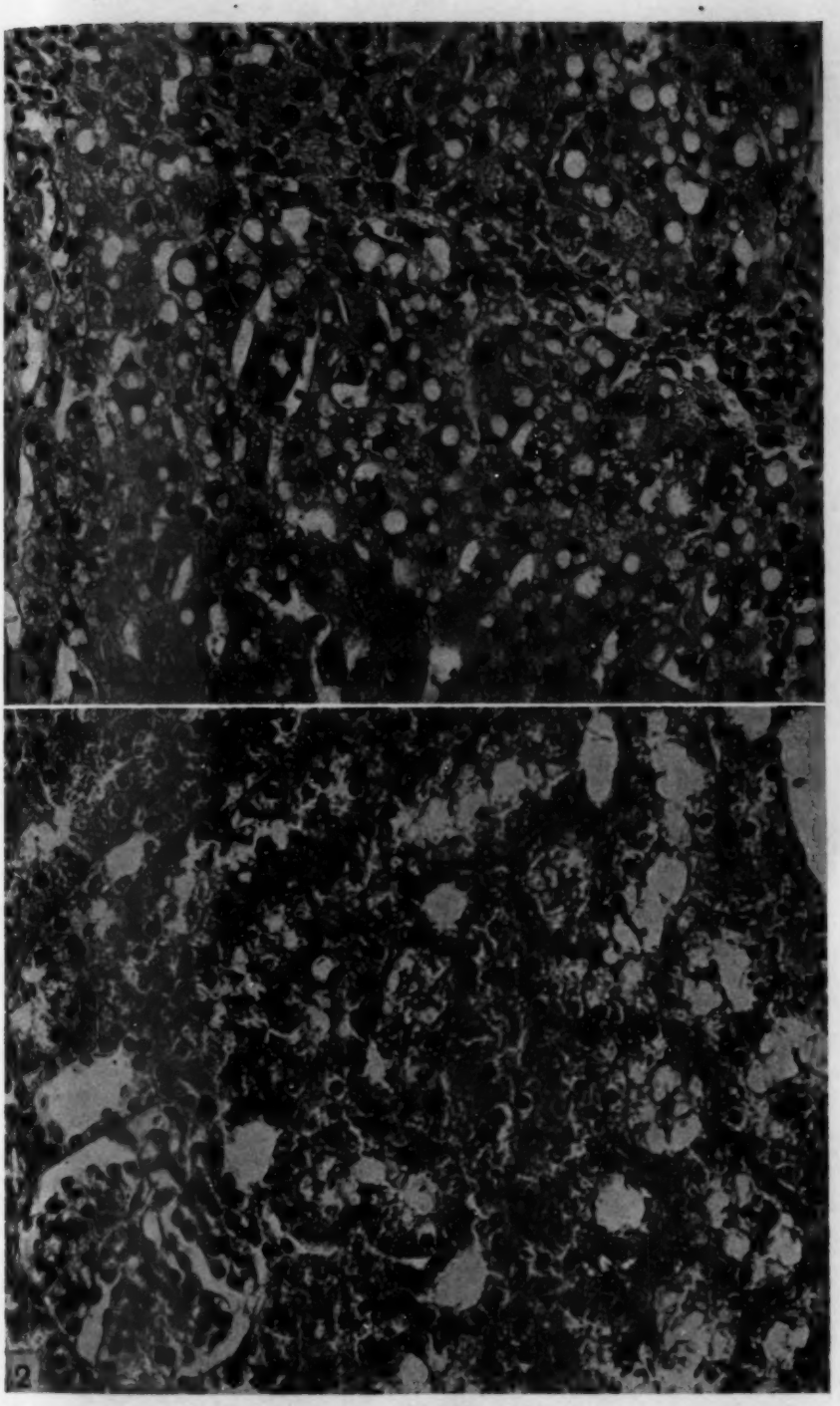

Fig. 1.—Photomicrograph of liver of rabbit 76; × 320. The rabbit received 60 mg. per kilogram of "stilbamidine" during three weeks.

Fig. 2.—Photomicrograph of kidney of rabbit 77; × 320. The rabbit received 60 mg. per kilogram of "stilbamidine" during three weeks.

fibrosis. The sinusoids were filled with blood. Glycogen was present in the liver cells. In the other animal, the liver was normal, and no fibrosis was present.

(*b*) Kidneys: Grossly, most of the kidneys looked normal, but some presented a pale cortex and a congested medulla. Histologically, the following features were observed: Rabbits which received doses of "stilbamidine" under 50 mg. per kilogram had some cloudy swelling in the convoluted tubules; in rabbits which received 50 mg. per kilogram in a five day period, the tubular cells were swollen and granular or reduced to fringes. Some glomeruli were swollen and enlarged.

In rabbits which received 60 mg. per kilogram during two weeks, cloudy swelling of convoluted tubules was extensive. The cells were either large and swollen and had very granular cytoplasm or were reduced to a narrow fringe. Staining with sudan III showed some fat in tubules, but only in a few cases was a large amount of fat observed.

With 75 mg. per kilogram the picture was about the same; cloudy swelling of various degrees was seen in the convoluted tubules and in the loops of Henle, while in the large collecting tubules the cells were very large and showed a rather vacuolated cytoplasm (fig. 2).

In rabbits which received 100 mg. per kilogram, either the tubular cells were so swollen that they entirely filled the lumens of the tubules or they were reduced to a narrow fringe. Glomeruli were often involved. When a glomerulus was involved, the tuft of capillaries was enlarged and reached the capsule in several places. In a few animals the glomeruli filled the entire subcapsular space. The glomeruli were often of different sizes in the same region.

In several animals many of the intertubular vessels of the medulla were intensely congested and enlarged. In some rabbits we observed the presence of arterioles with great hyperplasia of the intima and of the media. This was probably a manifestation of old arteriolosclerosis. Hyaline and sometimes granular casts were often noted, especially in the lower part of the medulla.

EXPERIMENTS ON MICE

The same organs which were examined in rabbits have been studied in mice. "Stilbamidine" was given by stomach tube or by subcutaneous injection. The mice were killed after one, two, three or more weeks of treatment. Doses of 10, 50 and 100 mg. per kilogram were given by mouth.

Mice given a subcutaneous injection of 50 mg. per kilogram daily died shortly after the doses totaled 550 mg. per kilogram. If death did not occur in a few hours after this total was reached, the mouse survived and never showed a delayed effect.

The changes observed after a short treatment of one or two weeks were dilatations of central and neighboring hepatic sinusoids and slight fatty metamorphosis, generally at the periphery of the lobules. After a long treatment, slight infiltration around the periphery of the lobules was observed, but it was never pronounced. Large cells whose cytoplasm was deeply stained with eosin were sometimes found. The size and the staining reaction of the nuclei of the hepatic cells were variable. In a few cases, isolated necrotic cells were noted (fig. 3).

The kidneys of the mice showed cloudy swelling as did those of the rabbits. The cells were swollen; many of the tubules were closed and often were filled with hyaline casts. In a few cases the glomeruli were swollen so as to occupy the entire subcapsular space (fig. 4). It is difficult to establish any relation between the dosage used and the characteristic appearance of the kidneys.

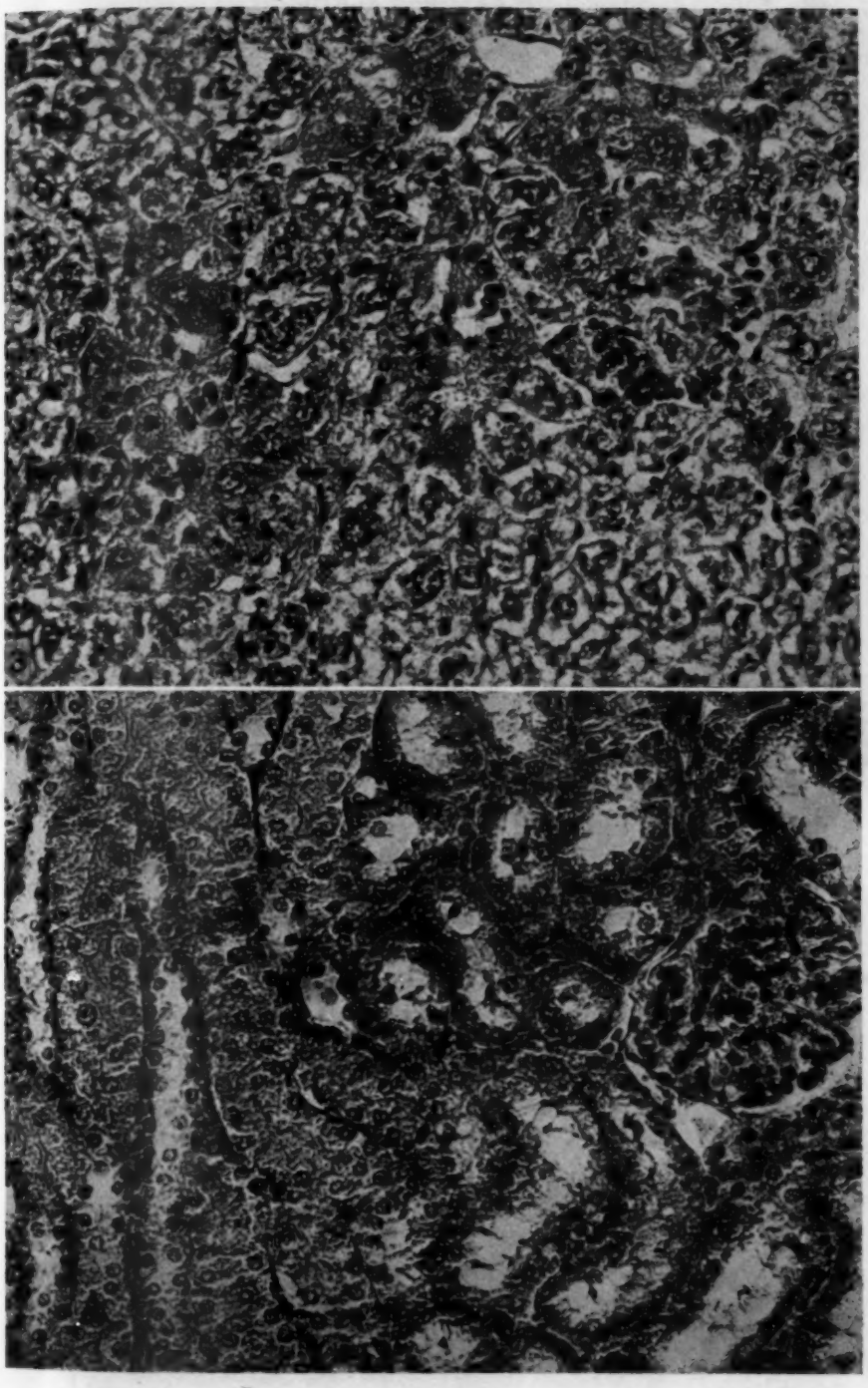

Fig. 3.—Photomicrograph of liver of mouse 70; × 320. The mouse received daily, by stomach tube, 10 mg. per kilogram of "stilbamidine" for a month.

Fig. 4.—Photomicrograph of kidney of mouse 70; × 320.

COMMENT

Our results compare in some respects with the results reported in the literature.

Large doses of aliphatic amidines, diamidines and diguanidines have been shown to affect the blood sugar and the blood urea nitrogen.[6] These changes have been correlated with hepatic damage.

Devine [4c] found that daily doses of 5 mg. per kilogram of "stilbamidine" given to rabbits by intravenous injection for six days did not produce any change in blood sugar or blood urea but that a single dose of 15 mg. per kilogram injected intravenously produced a sharp rise in blood sugar for a few hours, the concentration returning to normal in three days. A dose of 25 mg. per kilogram injected intravenously caused a short collapse, with the blood sugar rising to double the normal level.

In our experiments the blood sugar showed a variable response. There was often preliminary hypoglycemia, occurring in three to four weeks. This was followed by hyperglycemia, with an occasional return to a hypoglycemic level. The terminal blood sugar level was not always consistently elevated or depressed. In the experimental group the blood sugar varied from 52 to 202 mg., while in the controls it varied from 85 to 132 mg. per hundred cubic centimeters.

The average of the blood urea values was above the control level and fluctuated from 10.97 to 30.33 mg.

Other authors have studied the effects of the diaminides on various organs.

Gross and microscopic lesions produced by "propamidine" given to cattle have been described by Daubney and Hudson.[4d] They noted that marked fatty degeneration was produced in the liver.

Kirk and Henry [4e] found that the livers of animals receiving injections of "stilbamidine" presented fatty degeneration, accompanied by congestion. This fatty change occurred after fifteen minutes of an intravenous injection.

Wien, Freeman and Schotcher [3b] observed marked degeneration of the liver in healthy guinea pigs receiving 20 mg. per kilogram daily for five days. Doses of 4 and 8 mg. per kilogram given daily for five days produced no observable change in the liver, while in rabbits receiving 20 mg. per kilogram intravenously daily for six days they found moderate fatty degeneration of the liver.

Allen, Burgess nad Cameron,[4f] studying the toxic effect of "propamidine," observed hepatic and renal damage. They found that the production of necrosis of the liver was variable, sometimes affecting the peripheral zone of the lobules and sometimes the central zone.

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6. Seager and Rigdon.[3c] Bischoff, Sayhn and Long.[4a] Devine.[4b]

In our experiments the peripheral zone of the lobules was generally affected. Large masses of infiltrating cells and proliferated small bile ducts were encountered at the periphery of the hepatic lobules when the rabbit received at least 75 mg. per kilogram over a period of three or more weeks. Fibrous infiltration is always more noticeable in animals which survive the treatment for at least a few weeks. This infiltration resembles the precirrhotic changes seen in various kinds of poisonings. None of our controls and none of the rabbits which received small doses showed such pronounced infiltration as rabbits which received large doses and were killed after several weeks. It has to be remembered that often adult rabbits show some infiltration at the periphery of the lobules and that many of the livers of healthy rabbits are diseased. It is therefore difficult to say whether, or to what extent, this infiltration is produced by the action of the drug. Scattered necrosis was sometimes observed and generally occurred at the periphery of the lobules.

Acute phosphorus poisoning is known to produce fatty degeneration and necrosis of liver cells in rabbits; in phosphorus poisoning of longer duration, some interstitial fibrosis and proliferation of bile ducts are obtained. These changes occurred in our animals, but since we never observed any real distortion of the regular pattern, we cannot speak of precirrhotic changes.

In poisoning with carbon tetrachloride, Cameron and Oakley [4h] found a definite increase in the number of some of the small bile ducts.

Kirk and Henry,[4e] reporting a study of the toxicity of "stilbamidine," said that extensive fatty degeneration was the most constant and typical change observed in the liver. In our experiments, extensive fatty degeneration was never found. The changes were limited to the periphery of the lobules.

Cells containing fine brownish-pigmented granules were generally noted at the periphery of the lobules among the infiltrating cells in the livers of rabbits which had been treated with "stilbamidine." An iron-free pigment was described by Lubarsch (1899) [5a] as found in necrotic liver cells.

Kirk and Henry [4e] also reported intense congestion of the kidneys with all degrees of tubular nephritis and areas of hemorrhage in the medulla as the consequence of administration of "stilbamidine." Our experiments on rabbits produced cloudy swelling of various degrees in the convoluted tubules and in the loops of Henle. The glomeruli were sometimes involved. In several animals, many of the intertubular vessels of the medulla were intensely congested and enlarged. Hyaline and sometimes granular casts have been found, especially in the lower part of the medulla.

It was observed that mice were more resistant to "stilbamidine" than rabbits, and that rabbits presented a delayed effect of the drug.

The livers of the rabbits showed heavier infiltration at the periphery of the lobules than the livers of the mice. Cloudy swelling of the convoluted tubules was found in the kidneys of mice, and hyaline casts were common.

Recently Oasther and Fidler [46] found cerebral lesions in 10 of 20 dogs treated with "stilbamidine," 1 mg. per kilogram being given daily during the first week, increasing to 1.5 mg. and to 2.0 mg. per kilogram during the second and the third week, respectively. In 2 animals, microscopic lesions of the brain stem, the cortex and the spinal cord were observed. In all except one of these dogs the liver was normal. Slight fatty degeneration was observed in the loops of Henle.

SUMMARY

"Stilbamidine" was administered subcutaneously to rabbits and subcutaneously or by stomach tube to mice to determine the morphologic and functional changes that might be caused by chronic intoxication with this drug. The changes, mainly degenerative, occurring in the organs most affected, namely, the liver and the kidneys, are described in detail.

HAIR GROWTH IN THE SKIN OF GUINEA PIGS PAINTED WITH 20-METHYLCHOLANTHRENE

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AND
RUTH SILBERBERG, M.D.
ST. LOUIS

PROLONGED application of 20-methylcholanthrene produced in the skin of guinea pigs mild hyperplasia of the epidermis with increased mitotic proliferation of the basal cells. Although there were scattered hairless areas in the painted skin, growth of hair was rapid.[1] The present report deals with the histologic changes observed in hairs of the painted skin of these animals.

MATERIAL AND METHODS

The skin of 32 guinea pigs used in a former investigation of the effect of methylcholanthrene was studied.[1] In brief, the right ears and flanks were painted with a 3 per cent solution of 20-methylcholanthrene (dissolved in benzene) for periods of one-half, one, two, or three months. To the left ears and flanks of 16 of these animals benzene was applied; the left ears and flanks of the remaining 16 guinea pigs were not treated. Representative areas of the right side served as tests, and corresponding areas of the left side were used as benzene-treated or untreated controls.

Hair shafts and bulbs were counted in a standard area representing a low power field measuring 1.5 mm. in diameter, and averages were established on the basis of 100 fields counted in each animal. Mitoses were counted in the matrix of the hair (bulb) and the epithelium of the follicle. For this purpose, 1,000 sections of each, bulbs and shafts, were examined in ears and flanks of each guinea pig.

HISTOLOGIC EXAMINATION

In untreated skin, the hair was fairly uniformly spaced, and between the individual hairs the surface epithelium showed a more or less straight horizontal borderline toward the underlying cutis (fig. 1 *A*). The skin of the ear contained 3, and that of the flank 7, viable hairs in the standard area (table 1). Vegetating and dead hair *(Kolbenhaare* [2]*)* were scarce. The ratio of bulbs to hairs was

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From the Snodgras Laboratory of Pathology, City Hospital.

This study was aided in part by a grant from the United States Public Health Service (National Cancer Institute).

1. Silberberg, M., and Silberberg, R.: Arch. Path. **43**:143, 1947.

2. Pincus, F.: Die Anatomie der Haut, in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1927, vol. 1, pt. 1, p. 1. Danforth, C. H.: Hair, in Malisoff, W. M.: Dictionary of Biochemistry, New York, Philosophical Library, Inc., 1943.

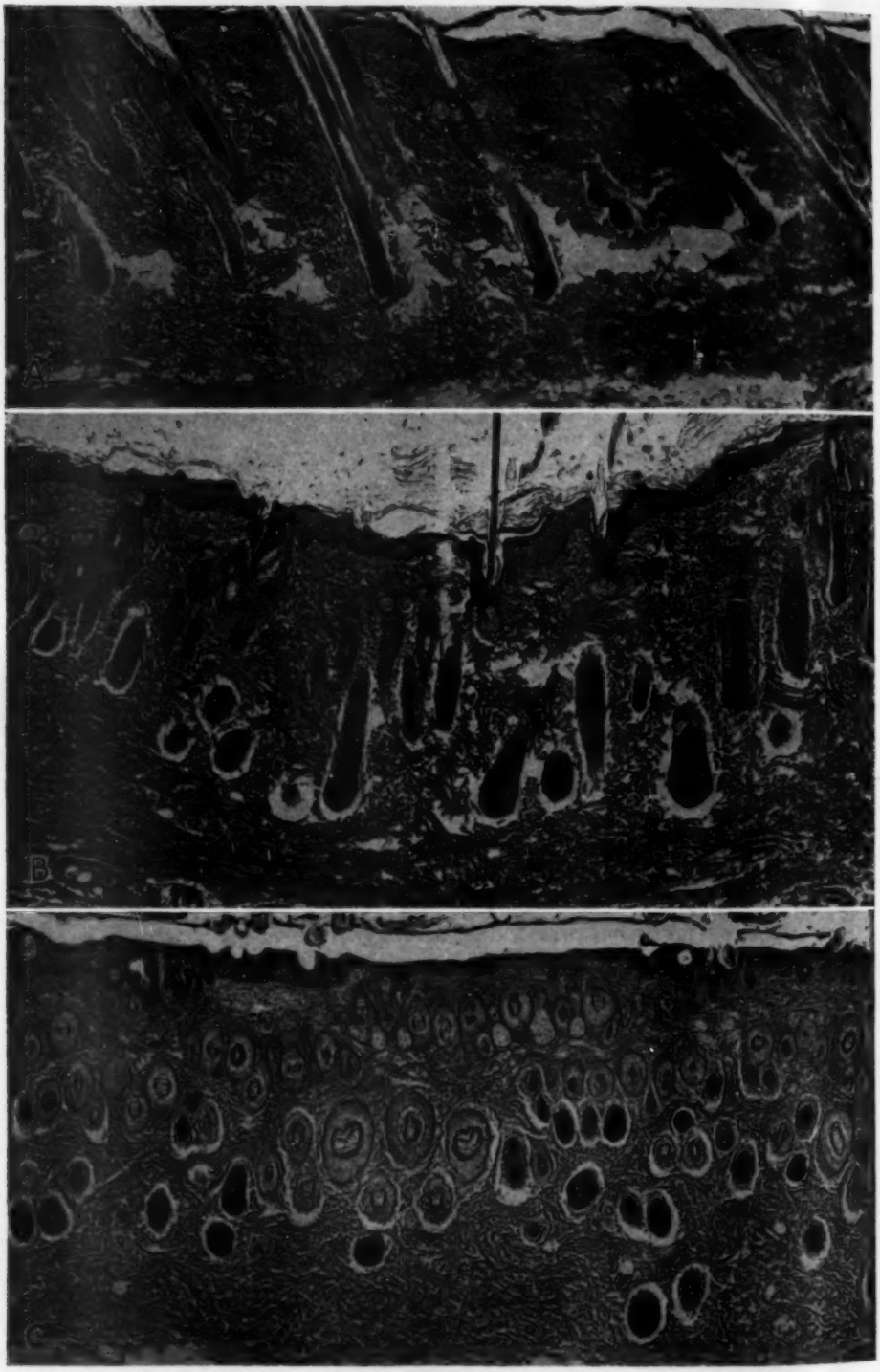

Figure 1

(See legend on opposite page)

10:11. Mitoses were more numerous in the matrix of the hair than in the epithelium of the follicle. The approximate numbers of mitoses per 2,000 cells (table 2) were: in the ear, 1.5 in the matrix and 0.5 in the epithelium of the follicle, compared with 3.5 in the surface epithelium; in the flank, 3.3 in the matrix and 1.1 in the epithelium of the follicle, compared with 2.5 in the surface epithelium.

After painting with benzene for one-half or one month, the hairs were more numerous and their distribution was more irregular than ordinarily (fig 1 *B*). They were definitely arranged in groups of three or four. The bulbs were enlarged, and some of them were situated somewhat more superficially than any in the nontreated control animal. Moreover, the slightly hyperplastic and hyper-

TABLE 1.—*Numbers of Viable Hairs and Epithelial Cords in a Lower Power Field*

Condition of Skin	Ear		Flank	
	Hairs	Cords	Hairs	Cords
Normal	3	.....	7	.....
Benzene painted				
½, 1 mo.	4	.....	12-13	2-3
2, 3 mo.	3-4	.....	12	.....
Methylcholanthrene painted				
½, 1 mo.	4-5	2-3	12-14	8-9
2, 3 mo.	4-5	1-2	13-14	3-4

TABLE 2.—*Number of Mitoses in Two Thousand Cells and in Multiples of the Normal (n) in the Matrix of the Hair, in the Epithelium of the Follicle and in the Surface Epithelium* *

Condition of Skin	Ear			Flank		
	Matrix	Follicle	Surface	Matrix	Follicle	Surface
Normal	1.5 (n)	0.5 (n)	3.5 (n)	3.3 (n)	1.1 (n)	2.5(n)
Benzene painted						
½, 1 mo.	4.3 (3n)	1.8 (3½n)	5.2 (1½n)	6.0 (2n)	2.2 (2n)	3.8 (1½n)
2, 3 mo.	4.2 (3n)	1.4 (3n)	5.3 (1½n)	2.9 (n)	1.0 (n)	3.5 (1½n)
Methylcholanthrene painted						
½, 1 mo.	5.4 (3½n)	2.8 (5½n)	5.4 (1½n)	6.5 (2n)	2.8 (2½n)	5.2 (2n)
2, 3 mo.	4.3 (3n)	1.5 (3n)	5.9 (1½n)	3.0 (n)	1.5 (1½n)	3.8 (1½n)

* The data for the surface epithelium are taken from the previous investigation and are added here for comparison.

trophic surface epithelium began to bulge into the thickened and congested dermis. The number of viable hairs of the standard area had increased to 4 in the ear and to 12 or 13 in the flank (table 1). Besides, there were in the latter 2 or 3 epithelial cords invaginating from the surface epithelium into the dermis. The

EXPLANATION OF FIGURE 1

A, normal skin of the flank (low magnification).

B, skin of the flank painted with benzene for one-half month (magnification as in *A*). The cutis is thickened; the hair follicles and bulbs are increased in number and size.

C, skin of the flank painted with methylcholanthrene for one-half month (magnification as in *A*). The cutis is thickened and contains a large number of vegetating or dead hairs and many hypertrophic bulbs.

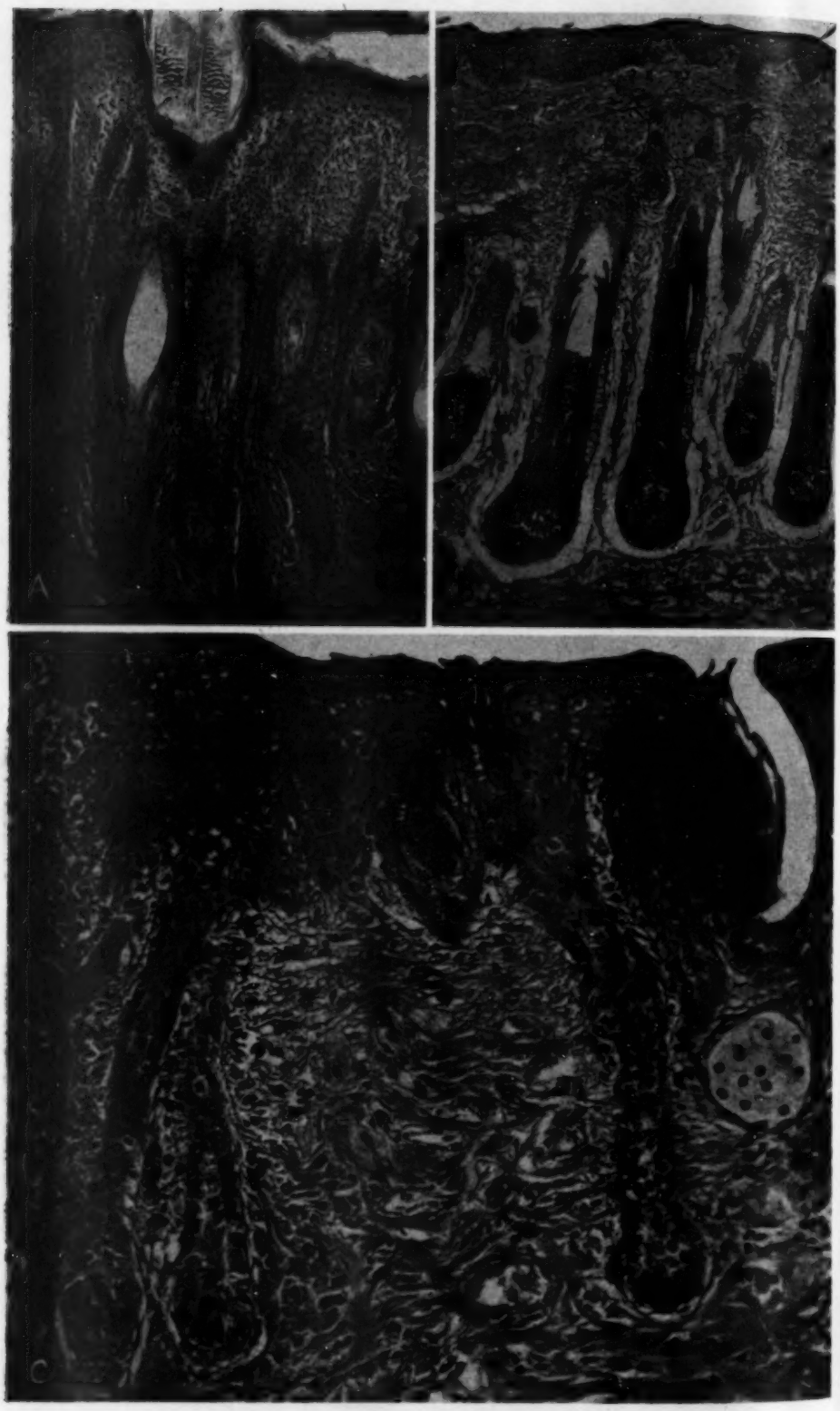

Figure 2

(See legend on opposite page)

sebaceous glands were unmistakably enlarged. The ratio of bulbs to hairs had changed to 10:16. Many hairs were thicker than usual; many were well developed. Some representing vegetating or dead hair showed regressive changes, such as atrophy or swelling and hyalinization. The epithelial cells of the hair follicles were increased in number and size: In the ear there were 4.3 mitoses in 2,000 cells of the matrix (i.e., three times the normal figure) and 1.8 mitoses in 2,000 cells of the follicle (i.e., three and a half times the normal value). In the flank, 6 mitoses were found in 2,000 cells of the matrix and 2.2 mitoses in 2,000 cells of the follicle (i.e., twice the normal in both locations). After prolonged application of benzene the number of hairs (table 1) did not materially differ from that found at the earlier experimental stages. In the ear the mitoses remained at approximately the same level, whereas in the flank the mitoses in both the matrix and the epithelium of the follicle had fallen to normal (table 2).

Methylcholanthrene stimulated the growth of hair more than benzene did. After one-half or one month of painting, the hairs again were arranged in groups, but the grouping was less distinct than in the benzene-treated animals because of the further increase in the number and the thickness of the follicles (fig. 1 *C*) and the epithelial pegs; 4 or 5 viable hairs and 2 or 3 epithelial cords were counted in the ear, and 12 to 14 viable hairs and 8 or 9 epithelial cords in the flank (table 1). However, in contrast to conditions in normal or benzene-treated animals, there was a large number of vegetating and dead hairs (fig. 1 *C*) in the dermis. In some areas the bulbs were again situated closer to the surface than ordinarily (fig. 2 *B*). Not infrequently, 2 hairs were seen to emerge from the same follicular opening (fig. 2 *A*), but in many areas the hairs were broken off at the level of the skin surface (fig. 1 *C*). The cells of the matrix and of the follicle had increased in number and size, and more so than had those in the guinea pigs painted with benzene. Regressive changes, in particular swelling and keratinization of the hair, were more accentuated and more widespread than after application of benzene. The hyperplastic and hypertrophic surface epithelium showed more numerous and deeper invaginations of the cutis as compared with that of the benzene-treated animals (fig. 2 *C*). Moreover, solid strands of epithelium budded out from the epithelium of follicles, usually from an area just above the origin of the sebaceous glands. These epithelial cords varied in length (fig. 3) and thickness, and many showed mitotic proliferation (fig. 3). The ratio of bulbs to hairs was 10:19. In the ear (table 2), there were 5.4 mitoses in 2,000 cells of the matrix (three and a half times the normal) and 2.8 mitoses in 2,000 cells of the follicular epithelium (five and a half times the normal). In the flank (table 2) the corresponding figures were 6.5 mitoses in 2,000 cells of the matrix (twice the normal) and 2.8 mitoses in 2,000 cells of the follicular epithelium (two and a half times the normal). After two or three months of application of methylcholanthrene there was no appreciable change in the number of hairs as

EXPLANATION OF FIGURE 2

A, skin of the flank painted with methylcholanthrene for one month (high medium magnification). The surface epithelium is hyperplastic, invaginating into the dermis at numerous points. Two hairs emerge from one follicular opening.

B, skin of the flank painted with methylcholanthrene for one-half month (low medium magnification). Note the small bulb close to the surface between the two sebaceous glands.

C, skin of the flank painted with methylcholanthrene for one-half month (high magnification). Several epithelial cords in the dermis show at their tips beginning formation of new bulbs and new dermal papillae.

Fig. 3.—Skin of the flank painted with methylcholanthrene for one-half month (high magnification). There is a long epithelial cord at the left side of the picture showing beginning bulb formation at the tip. An epithelial cord is budding out from the epithelium above the sebaceous gland. Note several mitoses in the epithelium.

compared with the numbers recorded at shorter stages, whereas both the growth processes and the regressive changes were less pronounced: In the ear 4 or 5 hairs and 1 or 2 epithelial cords, and in the flank 13 or 14 hairs and only 3 or 4 epithelial cords, were found in the standard area (table 1). Moreover, the mitotic counts (table 2) had declined: In the ear there were 4.3 mitoses in 2,000 cells of the matrix (three times the normal) and 1.5 mitoses in 2,000 cells of the epithelium of the follicle (three times the normal); in the flank the numbers of mitoses had dropped to normal or to one and a half times normal values, respectively.

COMMENT

In the skin of young guinea pigs, benzene and, more intensively, methylcholanthrene stimulated hair growth: The number of viable hairs was increased, particularly in the dermis of the flank; there were hyperplasia and hypertrophy of the epithelium of the follicles and of the matrices of the hairs. These findings are in agreement with the acceleration of hair growth observed grossly by Mottram [3] and by Butcher [4] under the influence of benzene or other irritants. Mitotic activity was stimulated in the follicles and in the matrices of the hair but less so in the flank than in the ear. Correspondingly, a return to normal was accomplished more readily in the former than in the latter. The stimulation of hair growth as indicated by the number of mitoses appearing in the matrices and the follicles did not progress, however, with prolonged application of methylcholanthrene. A peak was reached after one-half month. Thereafter the growth processes remained stationary or showed decline. Associated with this intensification of hair growth were regressive changes. These consisted of atrophy or of swelling and hyalinization of the hair, and many hairs were broken off near the surface. Again, the degenerative processes were more accentuated after application of methylcholanthrene than after that of benzene.

These results suggest a number of questions: (1) Is the increase in the number of hairs which was observed due to accelerated growth and replacement within the same follicle or (2) is there actual new formation of hair follicles? (3) Why does the epithelium of the hair follicles of the ear respond more vigorously to the growth stimuli than the surface epithelium of the ear? (4) What are the reasons for the difference in response of the epithelium of the ear and the flank?

Any increase in the number of viable hairs such as was observed under the influence of methylcholanthrene as well as under that of benzene cannot be discussed without touching on the moot question of whether or not new dermal papillae can be formed in postnatal life. Accelerated growth of the individual hair alone cannot account for the presence of a larger number of viable hairs. If one denies the new

3. Mottram, J. C.: Nature, London **155**:729, 1945.
4. Butcher, E. O.: Am. J. Physiol. **129**:553, 1940.

formation of papillae, one has to postulate accelerated formation of new hairs from the same papilla, possibly associated with delay in the shedding of the hairs. Under the influence of the growth stimuli the proliferation in the matrix may become so intensified that a new hair is formed immediately after the old one becomes detached from the papilla, without a period of rest such as normally occurs. The vegetating hair is still situated in the cutis as a new one or several new ones are being formed from below. This assumption seems to be corroborated by the findings of 2 hairs emerging from the same follicle (fig. 2*A*). Such an occurrence would constitute a relative delay of the shedding of old hair as compared with the rate of hair growth. But the shedding of the hair may also be absolutely retarded. The degenerating, hyalinized hairs might oppose greater resistance to the forces expelling them from the skin than the normal hair and thus stay inside the dermis longer than ordinarily. Degeneration of the hair is probably also the cause of the breaking-off of many of the hairs, which accounts for the hairlessness of some areas of the skin surface. This condition, thus, does not represent true epilation, since the corresponding areas of the dermis contain numerous viable hairs. It is also possible that the increased keratinization of the surface epithelium and the swelling and fibrillation of the connective tissue of the dermis caused by the irritants may render the extrusion of the old hair more difficult.

There are, however, indications that new hair may actually be formed from epithelium budding out from the follicles or from the surface epithelium of the skin. This mode of origin would necessitate the development of new bulbs including new cutaneous papillae such as occurs in embryonal or early postnatal life. Many epithelial pegs were found arising usually from the surface epithelium near the origin of sebaceous glands (fig. 2*C* and 3). These epithelial cords varied in length, but quite a few showed at their dermal ends a small club-shaped thickening suggesting the new formation of a hair bulb. Some of these structures were situated close to the surface (fig. 2*B*), and it seems plausible to assume that under the influence of epithelium growing out of the follicle new dermal papillae may develop. Even though new papillae may not develop in postnatal life under ordinary conditions, such new formation may occur under the influence of potent growth stimuli such as the polycyclic hydrocarbons. This conclusion should, however, be tested in further investigations.

A comparison of the growth processes of the epithelium of the matrix and the follicle with those of the surface epithelium shows differences in the degree of stimulation: Hair was more stimulated by both methylcholanthrene and benzene than was the epidermis. Benzene applied for a half or one month increased the number of mitoses three and a half times in the hair matrix of the ear but only one and a half

times in the surface epithelium. In the hair matrix of the flank the increase was twofold, compared with an epidermal increase of one and a half times the normal. Painting with methylcholanthrene for the same period multiplied the mitoses in the hair matrix of the ear three and a half times, compared with one and a half times in the epidermis; in the flank, on the other hand, the degree of mitotic proliferation was twice the normal in both hair matrix and surface epithelium. As seen from table 2, the stimulation of mitotic activity was even greater in the epithelium of the follicle (columns 4 and 7) than in that of the matrix of the hair (columns 3 and 6).

In mice painted with carcinogens, Mottram [5] observed similar differences in the response of surface and follicular epithelium and attributed them to a nonspecific factor, namely, the better vascular supply of the dermal structures as compared with the surface epithelium. Additional factors may, however, also be involved. There may be a selectivity of the applied stimulus for the hair and the hair follicle. In mice painted with methylcholanthrene the carcinogen is retained in the sebaceous glands.[6] In the guinea pig conditions may be similar. However, whereas in the mouse the hair follicles are destroyed by the carcinogen, in the guinea pig they react with marked stimulation of hair growth. Whether this difference in response is related to the failure of tumors to develop in the skin of the guinea pig under the influence of cyclic hydrocarbons has to be analyzed further.

There was a marked difference in response between the hair of the flank and that of the ear. Under the influence of the stimuli applied, there was in the flank a one and a half to two and a half fold increase in the number of mitoses, and the number of hairs increased 80 to 100 per cent. In the ear, on the other hand, a three to five and a half fold rise of mitoses was associated with but a 30 to 50 per cent increase in the number of hairs. Thus conditions in the ear must be less favorable to the formation of hair as compared with those in the flank in spite of the relatively lesser increase in the number of mitoses in the latter under conditions of stimulation. Moreover, this discrepancy indicates that new formation of hair is not simply a function of the mitotic activity of the epithelium but that other prerequisites have to be fulfilled before new hair is formed. One of the factors involved is the cyclic character of hair growth.[6] In hair matrices and follicles, phases of growth alternate with periods of rest. The high and low points of the cycle occur at different times in different regions of the body surface, and different responses may be elicited, depending on whether the stimulus acts on resting or on actively growing hair. Moreover, the

5. Simpson, W. L., and Cramer, W.: Cancer Research **3**:515, 1946.

6. Trotter, M.: Arch. Dermat. & Syph. **7**:93, 1923.

apparent regional differences might be accounted for by differences in types of hair. Five types of hair, varying in length, thickness and shape, have been found in the guinea pig.[7] It is possible that these types of hair differ not only in structure but also in growth rate. However, a true regional difference might be present, independent of the growth cycle and the type of hair and comparable to that found previously by Loeb[8] in the surface epithelium of the ear and of the flank in various animals: The epidermis of the ear is normally thicker than that of the flank, but the epithelium of the flank showed a more marked thickening under the influence of methylcholanthrene than that of the ear. In other words, there was a parallelism in the responses of surface epithelium and hair of each respective region: in the flank, marked thickening of the epidermis associated with marked increase in the number of hairs; in the ear, slight thickening of the epidermis accompanied by slight increase in the number of hairs.

Further investigations will have to answer more fully the questions arising from the present findings. Of special interest seems to be the inverse relationship between the growth stimulation of the hair and that of the surface epithelium. This condition exists not only in the guinea pig, which is resistant to the induction of cancer of the skin, but also in the mouse. In the latter, this inverse relation ends with tumors being formed in the epithelium after destruction of hair follicles.

SUMMARY

In young guinea pigs benzene and, still more intensely, methylcholanthrene stimulate hair growth: Mitotic proliferation and hypertrophy of the epithelium of the hair matrix and the hair follicle are increased, and there are indications that under the influence of the carcinogen new hair follicles and dermal papillae may be formed. This conclusion, however, should be tested in further investigations. Maximum growth is reached after approximately one-half month of treatment. Associated with the intensification of growth are regressive changes consisting of atrophy or swelling and keratinization of hair. The matrix of the hair and the epithelium of the follicle react to these experimental stimuli more actively than the epidermis. Hair growth is more stimulated in the flank than in the ear.

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7. Dawson, H. L.: Am. J. Anat. **45**:461, 1930.

8. Loeb, L., and Addison, W. H. F.: Arch. f. Entwcklngsmechn. d. Org. **37**:635, 1913. Loeb, L., and Spain, K. C.: J. Exper. Med. **25**:107, 1916; J. M. Research **41**:247, 1919.

ULTRASPECTROPHOTOMETRIC STUDIES OF EXTRACTS OF NORMAL AND OF TUMOR TISSUE OF HUMAN ORIGIN

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HISTOLOGIC properties in many instances have a chemical correlate traceable after the destruction of the cell structure. It seems highly probable that the great number of cells in malignant tumors, the frequent and atypical cell division and the hyperchromatism would become manifest in the extracts as an increase of the nucleic acids or of their cleavage products. Various authors (Claude and Rothen [1]; Thomas [2]; Stowell [3]) have even suggested that nucleic acids or their protein compounds may be an essential part of the active principle of tumor formation. When the properties of malignant growths are compared with similar properties of normal tissues or nonmalignant tumors, quantitative differences are to be expected (Davidson and Waymouth [4]; Schneider [5]), although qualitative ones may be possible. For the detection of nucleic acids, ultraspectrography has been used advantageously (Dhéré [6]; Spiegel-Adolf and Seibert [7]; Caspersson [8]; Spiegel-Adolf and Spiegel [9]). Our studies were therefore based on ultraspectrographic analyses of tissue extracts.

From the Department of Colloid Chemistry (Dr. Spiegel-Adolf) and the Agnes Barr Chase Cancer Research Foundation (Dr. Sano), Temple University School of Medicine and Hospital.

1. Claude, A., and Rothen, A.: J. Exper. Med. **71**:619, 1940.
2. Thomas, P. T.: Nature, London **156**:738, 1945.
3. Stowell, R. E.: Cancer Research **5**:788, 1945.
4. Davidson, J. N., and Waymouth, C.: Biochem. J. **38**:379, 1944.
5. Schneider, L. C.: Cancer Research **5**:717, 1945.
6. Dhéré, C.: Recherches spectrographiques sur l'absorption des rayons ultraviolets par les albuminoïdes, etc., Thesis, Fribourg, 1909.
7. Spiegel-Adolf, M., and Seibert, F. B.: J. Biol. Chem. **106**:373, 1934.
8. Caspersson, T.: Skandinav. Arch. f. Physiol., 1936, vol. 73, supp. 8. (*a*) Caspersson, T., and Schultz, J.: Nature, London **143**:602, 1939. (*b*) Caspersson, T., and Thorell, B.: Chromosoma **2**:132, 1942.
9. Spiegel-Adolf, M.; Spiegel, E. A.; Ashkenaz, E. W., and Lee, A. S.: J. Neuropath. & Exper. Neurol. **4**:277, 1945.

MATERIAL AND METHODS

Tissues were available from 30 patients. With the exception of 4 specimens, the material was obtained at operations. According to the outcome of clinical and histologic examinations and tissue cultures, 14 specimens were from malignant tumors, 1 was a lymph node from a patient with Hodgkin's disease, 1 was a lymph node from a patient with lymphatic leukemia and 3 came from benign tumors. Seven inflammatory lymph nodes and a specimen showing inflammatory hyperplasia of a breast gland, as well as 5 samples of various normal tissues, served as controls. One specimen came from a patient whose condition was clinically questionable and who is still under observation.

For the preparation of tissue extracts, the following technic was uniformly used: Approximately 1 cc. of tissue was finely cut with iris scissors and suspended in 3 cc. of isotonic solution of sodium chloride (p_H 7.2 to 7.4). The suspension was heated for one-half hour at 65 C. to inhibit the enzyme action (Greenstein [10]). After the suspension had been centrifuged at 2,000 rotations per minute for ten minutes, the volumes of the tissue and the saline solution were measured in the graduated centrifuge tube to establish the ratio between tissue and fluid extract. Sedimentation was induced by centrifuging the suspended material at 9,000 rotations per minute for thirty minutes in an angle centrifuge. The entire procedure was done under sterile conditions, and when, as a final stage, the extract was cultured in broth and on blood agar plate it was shown to be sterile except in 2 cases of postmortem specimens. The extracts were kept in the ice box between the single stages of preparation and when not in use.

For the ultraspectrophotometric studies a DU Beckman quartz spectrophotometer was available. It was used with a 5 mm. quartz cell. As most of the tissue extracts were opalescent, dilutions were made with isotonic solution of sodium chloride. According to a preliminary test, some of the solutions did not comply with the law of Beer (i. e., the extinction coefficients were not independent of the degree of dilution). It seemed therefore preferable to compare the solutions as to optical density in the same quartz cell at equal degrees of dilution. The measurements were made in steps of 10 angstrom units between 3,100 and 2,200 angstrom units. In some preliminary tests the effectiveness of the working conditions was ascertained. The heated extracts maintained their optical absorption power on standing in the ice box, while the unheated showed a marked decrease. Centrifuging at less than 9,000 rotations per minute for thirty minutes was not enough in clearing up the extracts. Centrifuging at 9,000 rotations per minute could be repeated for another period of thirty minutes without altering the optical absorption power of the tissue extracts. In contradistinction, 16,000 rotations per minute produced a marked decrease in the spectrophotometric results.

The optical studies of our tissue extracts gave the following results: Normal leukocytes when treated like the cell suspensions to which they were quantitatively comparable (equal length of centrifuged column) showed a faint indication of selective absorption at 2,600 angstrom units, but the graph looks rather S shaped within a wavelength range between 2,800 and 2,500 angstrom units. Hemolyzed blood, on the other hand, showed selective absorption only at 2,750 angstrom units, an optical behavior characteristic of the proteins.

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10. Greenstein, J. P.: The Biochemistry of Cancer, New York, Academic Press, Inc., 1947, p. 127.

Normal tissues of a high cell content show different spectrophotometric behavior. Liver is characterized by selective absorption with a peak at 2,650 angstrom units, confirming the findings of Carter and Greenstein.[11] Endometrium (late secretory stage) from a case of fibromyoma uteri showed similar results. The findings in the liver may be partly explained also by the role which, according to Barnes and Schönheimer,[12] this organ plays in the synthesis of purines and pyrimidines. The selective absorption of the endometrium is not surprising in view of its amazing regenerative capacity [12a] and its sensitivity to hormone stimulation. Further studies will be necessary to elucidate this problem. Seven inflammatory lymph nodes gave uniformly selective absorption, with the peak in 6 cases at 2,500 angstrom units. An inflammatory tumor of the breast gave, on the other hand, only an S shaped optical absorption graph. The results of the study of tumor extracts indicated likewise great uniformity. Of 10 carcinomas, 9 showed selective absorption, and only 1, a scirrhous carcinoma of the breast, showed an S shaped graph. Of the 9 specimens, 8 had a peak at 2,600 angstrom units, the height of which seemed in certain relation to the number of cells of the neoplastic tissue. This became clearly manifest in our 3 mammary tumors. Only 1 specimen, a medulloblastoma, was distinguished by two peaks, at 2,550 and 2,700 angstrom units. But it seems probable that the high lipid content of this preparation modified the properties of the tissue extract. Of 3 specimens of lymphosarcoma, 2 which were clinically and histologically similar gave nearly identical absorption graphs, with peaks at 2,600 angstrom units. The third, which gave an atypical histologic picture and which was diagnosed on the basis of the outcome of tissue culture, gave an atypical absorption graph likewise. A specimen from a patient with Hodgkin's disease gave findings similar to those noted with the 2 lymphosarcomas, while the absorption peak for tissue involved in chronic lymphatic leukemia was at 2,500 angstrom units as in the inflammatory nodes. Of the 3 benign tumors available, a fibromyoma and a hemangioma did not show selective absorption. One fibromyoma which was studied at nearly double the usual concentration showed a low peak at 2,550 angstrom units.

COMMENT AND CONCLUSIONS

As nucleic acids are normal constituents of tissues, it is obvious that differences in the characteristic absorption bands noticed with malignant tumors are primarily of a quantitative character. Our findings confirm those of Davidson and Waymouth,[4] according to whom tumor

11. Carter, C. E., and Greenstein, J. P.: J. Nat. Cancer, Inst. **7**:51, 1946.
12. Barnes, F. W., Jr., and Schönheimer, R.: J. Biol. Chem. **151**:123, 1943.
12a. Brues, A. M.; Tracy, M. M., and Cohn, W. E.: Science **95**:558, 1942.

tissue has usually a higher nucleic acid content than its tissue of origin. No explanation can yet be offered for the facts that the peak of selective absorption is about 2,650 angstrom units for normal liver and endometrium, 2,600 units for carcinoma and 2,500 units for inflammatory lymph nodes. Tentatively, it could be suggested as Lavin, Loring and Stanley [13] assumed for tobacco mosaic virus, and one of us (Spiegel-Adolf [14]) for cerebrospinal fluids, that selective absorption at 2,650 angstrom units is due to the influence of proteins on nucleic acids. The peak

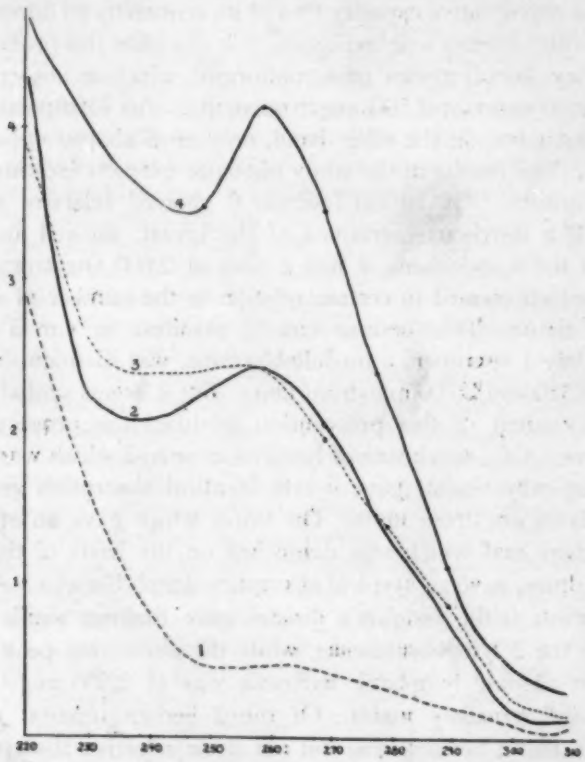

Fig. 1.—Optical absorption graphs of tissue extracts from various tumors of the breast (dilution of extracts, 1:4): *1*, scirrhous carcinoma; *2*, medullary carcinoma; *3*, inflammatory hyperplasia; *4*, medullary carcinoma, highly cellular type.

of 2,600 units for tumors could be explained by more extensive breakdown of nucleoproteins. In the presence of brain tumors cerebrospinal fluids may show similar selective absorption; this may be due, at least partly, not only to nucleoproteins or their derivatives but also to other

13. Lavin, G. J.; Loring, H. L., and Stanley, W. M.: J. Biol. Chem. **130**:259, 1939.

14. Spiegel-Adolf, M.; Wycis, H. T., and Spiegel, E. A.: J. Optic. Soc. America **35**:800, 1945; Federation Proc. **5**:156, 1946.

absorbing substances—for example, ascorbic acid (Spiegel-Adolf and Wycis [15]). The question why the absorption peak of inflammatory nodes is at 2,500 angstrom units will have to be made the subject of further studies. According to Vlès and Kagans,[16] the absorption peak of pure ribonucleic acid with p_H 4.7 is at 2,600 angstrom units. Shifts of this peak as far as 2,550 angstrom units have been observed by these authors between p_H 5 and p_H 10. Stimson and Reuter [17] were unable to observe a noticeable shift in the absorption peaks of either purified yeast or thymonucleic acids at various p_H values. It is questionable anyhow how far

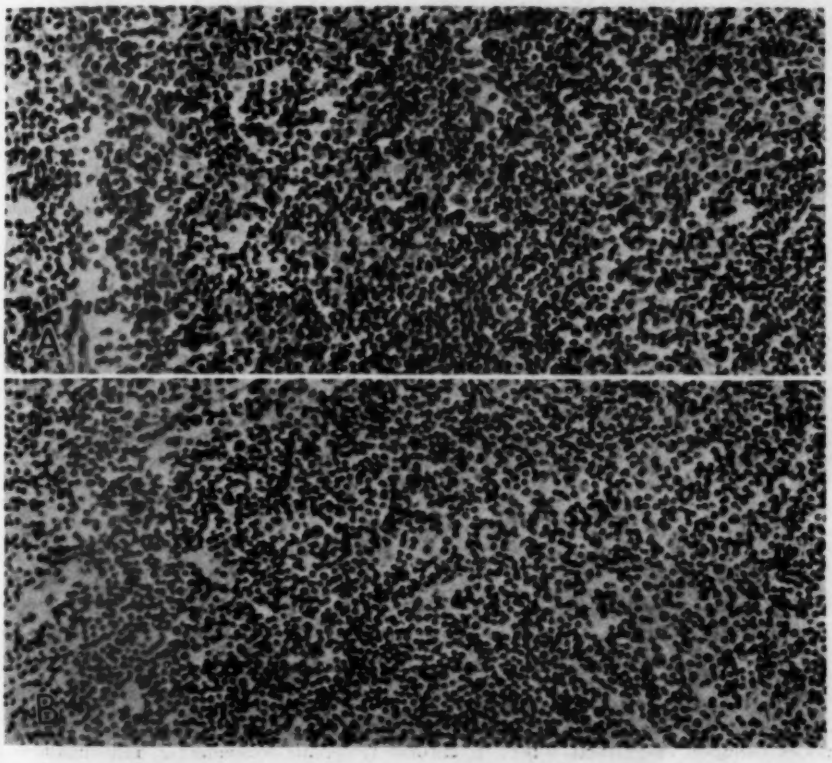

Fig. 2.—Histopathologic comparison of lymph nodes: *A*, lymphosarcoma; *B*, lymph node showing inflammatory hyperplasia. Hematoxylin and eosin stain; × 200.

changes in the hydrogen ion concentrations of inflammatory tissues become manifest in our tissue extracts. Nevertheless, a few practical conclusions may already be attempted on the basis of the present material. Our results allow us to differentiate between different kinds of

15. Spiegel-Adolf, M., and Wycis, H. T.: J. Phys. Chem. **50**:447, 1946.
16. Vlès, F., and Kagans, R.: Arch. de physique biol., 1943, vol. 16, supp. 110.
17. Stimson, M. M., and Reuter, M. A.: J. Am. Chem. Soc. **67**:847, 1945.

tumors of one organ, a finding which may be of prognostic importance (fig. 1). From the diagnostic point of view, the behavior of the lymph nodes under various pathologic conditions seems of interest. A count of cells alone does not explain the spectrophotometric behavior. Figure 2 shows the specimens equally rich in cells; one an inflammatory lymph node, the other a lymphosarcoma. Nevertheless, the peak of the extract of the first is at 2,500, the other at 2,600, angstrom units. Figure 3 visualizes those spectrographic differences, adding a specimen from a case of chronic lymphatic leukemia which shows optical absorption

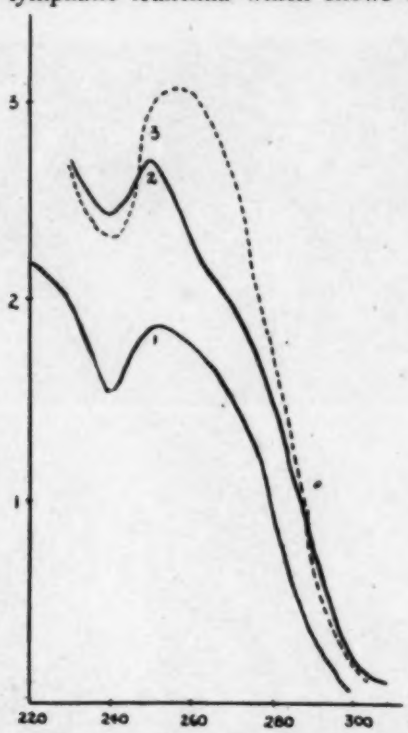

Fig. 3.—Optical absorption graphs of tissue extracts from pathologic lymph nodes (dilution of extracts, 1:9): *1*, chronic lymphatic leukemia; *2*, inflammatory hyperplasia; *3*, lymphosarcoma.

similar to that of inflammatory lymph nodes. It seems of interest in this respect that, besides the histologic likeness to the inflammatory lymph node, the antigenic properties of the leukemic lymphocytes and the normal ones cannot be distinguished.[18] Although no quanitative considerations are included, we wish to point to the fact that in figure 3 the highest peak is shown by lymphosarcoma. This may be expected from the nucleic acid content, while ascorbic acid, giving absorption in

18. Steinberg, B., and Martin, R. A.: Am. J. Path. 22:652, 1946.

the same range, seems to be less concentrated in lymphosarcoma than in normal lymph nodes, according to Greenstein's [10] observations regarding rats. Finally, we wish to mention a case which illustrates the specificity of our findings. A small tumor metastasis could be separated from the rest of a lymph node. Tissue extracts and microscopic slides were made from each part separately. The spectrophotometric findings confirmed the histologic ones, the tumor part showing an absorption peak at 2,600 angstrom units and the inflammatory lymph node part having its maximum absorption of 2,500 units.

SUMMARY

Ultraspectrophotometric studies of extracts of normal and of tumor tissue of human origin were made with the following results: Tissues from carcinoma and lymphosarcoma gave practically uniformly selective absorption peaks at 2,600 angstrom units. Extracts from inflammatory lymph nodes had their maximum of selective absorption at 2,500 units, while normal liver and endometrium had their height of absorption at 2,650 units.

Case Reports

LEIOMYOMA OF THE FEMALE BREAST

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THE LITERATURE contains fairly numerous references to a small, smooth muscle tumor arising from the nipple or the areolar region of the breast. The cases have been collectively reviewed by Melnick,[1] Driak and Sternberg,[2] Schauder [3] and Lindfors.[4] This tumor is generally considered to arise from the smooth muscle of the papillae mammae and the lactiferous ducts. There is a smooth muscle tumor of the breast proper, unrelated to the nipple or the skin, which is rare; its origin is equally obscure. Stein [5] admitted only 5 cases of this type; he excluded cases of myosarcoma and myoepithelial tumor.

REPORT OF A CASE

M. S., a 40 year old Negro woman, married, who had been pregnant twice but had borne only one full term, living child, had a mass in the left breast. This mass was first noted ten years previously, shortly after the birth of her only child. It remained stationary in size and was painless until three months before she entered the hospital. During the latter period the patient noted twinges of pain in the involved region in the course of her menstrual periods. There was no fluctuation in the size of the mass, nor were there any other symptoms related to the breasts. The patient had received antisyphilitic therapy during her pregnancy and again for the last two years before her hospitalization. On physical examination, the breasts were heavy and pendulous, and the left breast was definitely enlarged. The nipple and the areola were normal. In the inferolateral quadrant of the left breast, 9 cm. from the nipple, there was a sharply demarcated, resilient mass the size of a baseball. It was attached neither to the skin nor to the underlying muscle. The remainder of the left breast and the right breast were normal to palpation. The axillary lymph nodes were not enlarged. The blood Hinton reaction for syphilis was negative.

At operation a 5 cm. linear incision was made over the center of the tumor. The tumor was encapsulated and was shelled out from the rest of the breast along an easily definable plane of cleavage. No further resection of the breast was considered necessary, and the wound was closed in layers.

The mass was found to measure 10 by 8 by 4 cm. and to weigh 162 Gm. The external surface was smooth except where its ellipsoid shape was distorted by variously sized rounded protuberances having diameters up to 3 cm. These rose

From the Department of Pathology, Peter Brent Brigham Hospital.

1. Melnick, P.: Arch. Path. **14**:794, 1932.
2. Driak, F., and Sternberg, H.: Deutsche Ztschr. f. Chir. **207**:353, 1928.
3. Schauder, H.: Deutsche Ztschr. f. Chir. **205**:58, 1927.
4. Lindfors, A. O.: Monatschr. f. Geburtsh. u. Gynäk. **11**:763, 1900.
5. Stein, R. J.: Arch. Path. **33**:72, 1942.

above the general surface of the mass for distances of 0.5 to 2 cm. The surface of the tumor was a uniform pinkish white. On palpation the mass consisted of many rounded firm nodules of varying sizes set in a softer intervening tissue. Hemisection of the tumor showed numbers of white nodules rising above the general plane of

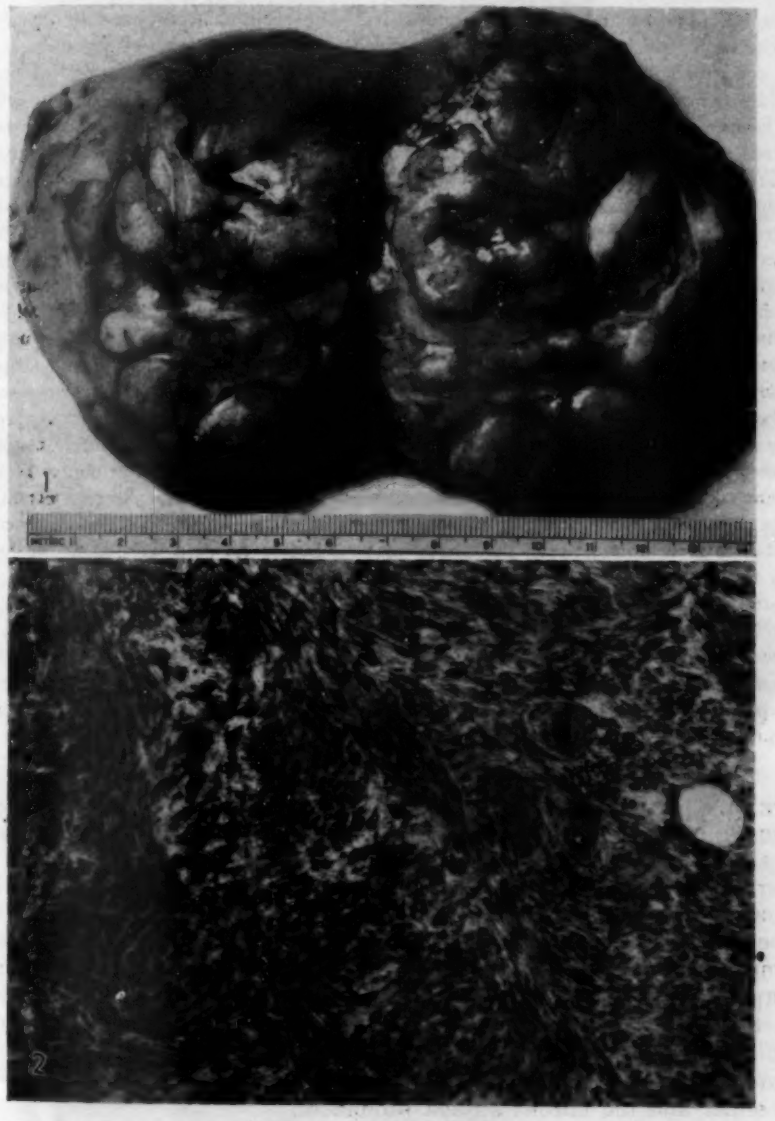

Fig. 1.—Cross section of the tumor showing the nodular character of the mass.

Fig. 2.—Microscopic section of the tumor showing the arrangement of the muscle bundles and their relation to the blood vessels. Eosin methylene-blue; X 106.5.

the cut surface for distances up to 0.5 cm. These nodules ranged from pinpoint-sized dots to irregularly rounded masses 3.0 cm. in diameter. The softer intervening stromal tissue was translucent, slightly rubbery in consistency and yellowish white.

Nine separate samples of the tumor were taken from distinctive portions of the specimen for microscopic examination. The sections were fixed in Zenker's solution to which acetic acid had been added to the concentration of 5 per cent and 4 per cent solution of formaldehyde and were stained with eosin–methylene blue, Mallory's aniline blue connective tissue stain and phosphotungstic acid–hematoxylin. The predominant feature of these sections was the presence of numerous variously sized groups of smooth muscle cells having a closely related and intimately mixed fibrous tissue stroma. The smooth muscle cells appeared in wavy continuous bundles, five to six cells in width, and as single cells almost isolated by the fibrous stroma cells. The smooth muscle cells were characterized by elongate regular nuclei with rounded ends and prominent small clumps of chromatin, and by longitudinal myoglial fibrils. These fibrils took a deep blue stain with phosphotungstic acid–hematoxylin and retained the acid fuchsin when stained with Mallory's aniline blue connective tissue stain. In comparison with these, the stromal cells had nuclei that were more vesicular, with much more variation in nuclear size and shape; some were almost round, while others were long and undulating. They were seen closely applied to collagen bundles. In some areas the tumor mass formed a capsule by compression of the surrounding tissue; yet in others the smooth muscle bundles faded off into a loose meshwork of edematous, acellular, fibrous interlobular breast tissue. In a few areas this interlobular tissue appeared to have become necrotic, the individual fibers breaking up and the nuclei disappearing. Normal mammary lobules were seen between the tumor masses, but in no case were they involved by the tumorous process; they were surrounded by a dense, hyalinized collagenous matrix. Among the smooth muscle bundles, the blood vessels were numerous, and the smooth muscle fibers could be seen curving about them. In some areas there was considerable hyperplasia of the fibrous layers of the smaller arterioles with distinct deposition of collagen between the cells; this was reminiscent of the hyperplasia of the adventitial layers of blood vessels seen in some meningiomas. In no case did the circular muscle fibers of the blood vessels take part in the tumor.

The reported cases of pure leiomyoma of the breast are gathered with the present one in the accompanying table. The 2 cases of smooth muscle tumor reported by Klob [6] and cited by Stein [5] are omitted from the table because the specimens are not adequately described. From a perusal of the table it is seen that leiomyoma of the breast occurs in women in the fifth and sixth decades. In all of the patients, however, the tumor had been present a long time before its removal; this fact emphasizes its slow rate of growth. In each instance the tumor was large and encapsulated, and in most cases, freely movable. In some instances it was of an almost uniform consistency, broken only by one or more cystic structures; in others it was made up of many nodules, variously sized. In the majority of the cases the tumor was quite vascular and the fibrous stroma prominent.

The origin of leiomyoma of the breast is still a matter of speculation. Melnick [1] suggested that it may arise (1) from a teratoid tumor

6. Klob, J. M.: Pathologische Anatomie der weiblichen Sexualorganen, Vienna, W. Braumüller, 1864, p. 492.

with an overgrowth of the myomatous elements, (2) from smooth muscle elements displaced from the nipple region, (3) from the myoepithelial layer of the ductal structure and (4) from the muscular portions of the blood vessels. He favored the latter explanation for its origin in the case which he described. The case described by Strong [7] lends support to this theory because of the intimate relation of the

Cases of Pure Leiomyoma

Author	Clinical Data	Size and Location	Gross Description	Microscopic Examination
Strong [7].....	46 yr. old woman; duration of tumor 4 yr., with rapid growth last 2 yr.; discomfort 6 mo.	6 x 3 cm. oval mass; distinct from nipple; attached to underlying fascia	Encapsulated, firm, fibrous and uniform grossly; 1 cm. high triangular cystic area in center	Bundles of smooth muscle fibers; blood vessels prominent; smaller blood vessels take an active part in tumor
Melnick [1].....	48 yr. old woman; duration of tumor 15 yr., with rapid growth last 2 mo.	"Grapefruit size"; 1 cm. from nipple but independent of it	Encapsulated, firm and elastic; replaces entire breast; 2 x 2 x 3 cm. cyst in center	Blood vessel muscular coat blends into tumor; muscle bundles have a perivascular arrangement; thrombosis of blood vessel near cystic area
Lebowitch, R. J., and Lenz, G.: Am. J. Cancer 38 : 73, 1940	58 yr. old woman; duration of tumor 17 yr.; not tender, freely movable	13.8 cm. in diameter; margin 2.5 cm. from nipple	Encapsulated, firm and fibrous; numerous minute cysts on cut surface	Smooth muscle cells with myofibrils and a well vascularized matrix of collagen; few blood vessels in muscle bundles
Stein [6].....	54 yr. old woman; duration of tumor 26 yr.; increase of size past 9 yr.; firm, circumscribed, freely movable	Nipple unconnected with tumor mass	Encapsulated; numerous nodules rising above cut surface	Mixed smooth muscle tissue and fibrous tissue; areas of undifferentiated connective tissue
This case.....	40 yr. old woman; duration of tumor 10 yr.; painful 8 mo.; no rapid change in size, freely movable	10 x 8 x 4 cm. ellipsoid mass; 9 cm. from nipple	Encapsulated, with palpable nodules; numerous nodules rising above cut section	Smooth muscle bundles with fibrous tissue matrix; well vascularized; blood vessels play no direct role in tumor formation

muscular structure of the blood vessels to the tumor proper. In this case the relations of the tumor to its blood vessels or other structures of the breast give no clue to its origin.

SUMMARY

A case of leiomyoma of the breast is described, and the similar cases are gathered from the literature.

7. Strong, L. W.: Am. J. Obst. **68**:83, 1913.

PERFORATION OF THE STOMACH OF A NEWBORN INFANT WITH PYLORIC ATRESIA

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THE PERFORATION of the stomach to be reported occurred in a 2 day old Negro infant in whom atresia of the pyloric portion of the stomach was discovered at necropsy. This is believed to be the seventeenth reported case [1] of perforation of the stomach of the newborn, the third of pyloric atresia and the first of pyloric atresia complicated by perforation of the stomach.

REPORT OF A CASE

A Negro boy was delivered at term by cephalic presentation after episiotomy and use of forceps on March 8, 1947, at 8:09 a. m. Polyhydramnios was present. The child cried spontaneously and appeared well formed. The mother was a 24 year old secundipara, and this had been her fourth pregnancy. The Mazzini test of her blood was negative; the Rh test was positive. At 7 p. m. of the same day the child's abdomen and scrotum became distended; no bowel movements had occurred. Digital examination of the rectum revealed no abnormality. Peristaltic sounds were not heard on auscultation of the abdomen. The child soon became dyspneic. Large amounts of air were discovered in the peritoneal cavity on fluoroscopic examination. At 2 p. m. the next day, exploratory laparotomy disclosed free air and about 60 cc. of turbid liquid in the peritoneal cavity. Near the greater curvature of the stomach there was a necrotic area, 2 cm. in diameter, in which perforation had occurred. The contour of the rest of the stomach was not unusual. The other organs appeared intact. The site of perforation was sutured with interrupted no. 00 chromic catgut, and the abdomen was closed. Supportive treatment, with dextrose and plasma administered intravenously, was of no avail, and the child died the next day, March 10, at 1:45 p. m.

Necropsy (one hour after death).—The body was 50 cm. long and weighed 2,255 Gm. There were no anomalies of the mouth. The neck and the chest were symmetric. The abdomen was slightly distended and somewhat tense. There was a recently sutured incision of the right rectus abdominis muscle, 5 cm. long with a 4 cm. medial transverse extension at the upper end. The scrotum was tense and waterlogged, and 3 cm. in diameter. The testicles were of the usual size.

The peritoneal cavity contained approximately 20 cc. of dark bloody fluid. The anterior surfaces of the liver, the stomach, the spleen, the transverse colon and the omentum were matted together by a fibrinous exudate. There were no anomalous positions of abdominal organs. There was no excess fluid in the pleural or the pericardial cavity. There were no anomalies of the heart and large vessels. Both lungs were air containing throughout.

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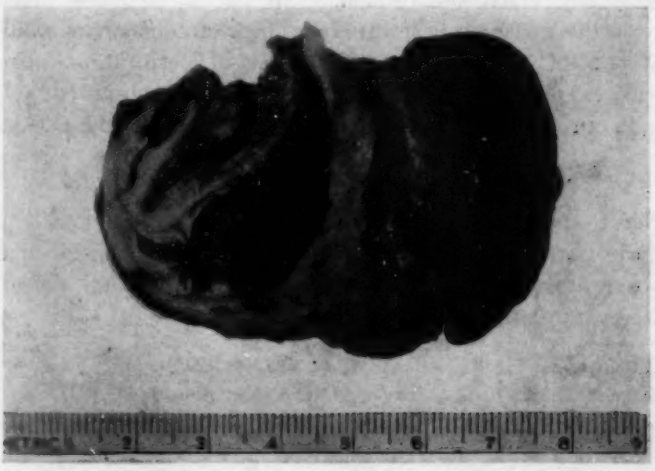
1. Herbut, P. A.: Arch. Path. **36**:91, 1943.

The stomach measured 6 by 3 cm. In the fundic portion, over the anterior surface, near the greater curvature there was an area of red-brown discoloration, 2 by 1 cm. In the pyloric portion for a length of 1 cm., there was a barely perceptible narrowing to 0.6 cm. in diameter. The mucosal surface was smooth, with the usual gyrations, except over the area of discoloration. In the center of this was the site of perforation, 1 cm. long. In the pyloric portion the lumen was interrupted for a length of 1 cm. The obstruction ended about 1 cm. proximal to the duodenum (figure). The wall of the duodenum was of the usual thickness; its mucosal pattern appeared intact. The loops of small intestine were collapsed and contained green pasty meconium. The colon was contracted and contained a small amount of viscid green liquid. No change was noted on the mucosal surfaces of the small and large intestines and rectum. The left kidney exhibited two complete pelves and ureters with corresponding openings into the urinary bladder. There were no other anomalies.



The segment of the pylorus lacking a lumen. It is about 1 cm. long and ends about 1 cm. proximal to the junction with the duodenum.

Microscopic sections of the stomach from near the site of perforation revealed good preservation of the gastric mucosa. In the tunica propria there were focal extravasations of blood. The submucosa was spread apart and infiltrated by red blood cells. Extensive extravasations of blood were seen in the muscular layers. Externally the area was covered by a hemorrhagic fibrinopurulent exudate. The muscle layers were fairly broad in the uninvolved portion; near the site of perforation they were thin. The appearance was essentially similar in three additional sections from deeper levels of the block.

In a section from the narrow portion of the pylorus, there was no lumen, and the mucosa and the muscularis mucosae were absent. The space was filled by vascular loose connective tissue like that of the submucosa. The appearance was essentially similar in six additional sections from deeper levels. Sections from the duodenum at the level of the papilla of Vater disclosed perfect preservation of the surface epithelium and no changes in the other layers.

COMMENT

Interruption of the continuity of the lumen of the intestine may be due to aplasia, as in Holladay's [2] patient, who lacked a segment 2 cm. long of the pyloric portion of the stomach. It may also be due to the presence of a membranous diaphragm, as in the case reported by Bennett [3] in which a diaphragm obstructed the pyloric portion of the stomach. A third kind of interruption of the lumen may be due to absence of the lumen, mucosa and muscularis mucosae being missing, with the other layers intact. In our case there was true atresia—that is, absence of the lumen of the pyloric portion of the stomach, with the other layers unaffected.

In none of the 16 cases of perforation of the stomach on record was there any distal obstruction.[1] In the 2 recorded instances of obstruction there was no perforation. These circumstances would eliminate mechanical obstruction as the cause of perforation. Congenital focal weakness of the muscular coats of the stomach, observed by Herbut [1] and also in our case, might be the predisposing cause of the perforation.

SUMMARY

Perforation of the stomach of a 2 day old Negro infant with atresia of the pyloric portion of the stomach is reported. The principal factor predisposing to perforation appears to be a congenital defect of the muscular coats of the stomach.

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2. Holladay, L. T.: J. Indiana M. A. **39**:350, 1946.
3. Bennett, R. J.: Am. J. Digest. Dis. & Nutrition **4**:44, 1937.

Laboratory Methods and Technical Notes

A FIBRINOGEN-THROMBIN CLOT USEFUL IN TISSUE CULTURE

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ONE OF the problems of culturing mammalian cells in vitro is that of insuring uniformity and sterility of the materials used in preparing the tissue and maintaining it in its container. Two substances commonly used are chicken plasma and chick embryo extract. These form the clot used to provide a solid substrate for the growth of the cells.

Chicken plasma is obtained usually by bleeding hens of standard weight and age. Carrel [1] found that the use of plasma from older hens results in poor growth of cultured cells. The plasma must be obtained with great care to prevent clotting. The procedure is tedious and invites many chances of infection. Chick embryo extract is subject to even greater variation and must be used within five to seven days if it is to produce a clot. Earle [2] adds horse serum to the mixture to help prevent liquefaction of the clot, as well as for its growth-promoting properties.

For the past year a mixture of purified fibrinogen [3] and thrombin [3] has been used to form a clot in preparing tissue cultures. The materials are relatively uniform and sterile, and they form a strong clot which will not liquefy for at least six weeks, even when the clot is filled with actively growing fibrocytes.

MATERIALS AND METHOD

The method of producing a fibrinogen-thrombin clot is as follows: The thrombin (labeled: 5,000 units, topical, bovine origin) is used in amounts of 10 mg. in 1 cc. of isotonic solution of sodium chloride as reported by Earle.[2] The purified fibrinogen is used in amount of 50 mg. in 1 cc. of isotonic solution of sodium chloride. The fibrinogen dissolves slowly and stirring should be avoided. The thrombin dissolves easily and quickly. Physiologic balanced solution of sodium chloride may be used instead of isotonic. Porter flasks [4] are used throughout. The body of the flask is 3.5 cm. in diameter and 1.5 cm. thick. Its neck is 16 cm. long and 1.5 cm. in diameter. One part of the thrombin solution added to 4 parts of the fibrinogen solution will form a clot in less than thirty seconds. Place the tissue to be cultured in position, add 4 to 6 drops of the fibrinogen solution and then add 1 or 2 drops of the thrombin solution. Within a few minutes the mixture will have formed a gel, and a supernatant layer of routine nutrient may be added freely. The amounts of the solutions of fibrinogen and

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1. Carrel, A.: J. Exper. Med. **18**:287, 1913.
2. Earle, W. R., and others: J. Nat. Cancer Inst. **4**:165, 1943.
3. This was supplied by Parke, Davis & Company.
4. Porter, K. R.; Claude, A., and Fullam, E. F.: J. Exper. Med. **81**:233, 1945.

thrombin may be approximate. Accurate weighing is unnecessary. The thrombin solution remains potent for twenty-four hours. The fibrinogen solution is usually clotted by standing overnight even in the cold. The powdered thrombin and fibrinogen remain effective indefinitely as long as they are kept dry. They can be kept in an incubator at 37 C. to prevent dampness. Care should be taken to prevent the solutions from becoming contaminated with each other, with plasma or with tissue juices, since the thrombin is quickly activated and the fibrinogen clots before it can be used.

The tissues cultured have been chiefly of human origin. They are placed in isotonic solution of sodium chloride and cut into small pieces. Each piece is then transferred to the surface of a single cover slip placed on one side of a Porter flask. A few drops of fibrinogen solution are placed on the piece of tissue, followed by a few drops of the thrombin solution. A few moments later, a few drops of a nutrient are placed in the flask, and a mixture of 5 per cent carbon dioxide, 21 per cent oxygen and 74 per cent nitrogen is poured into the flask to replace the air. The nutrient consists of equal parts of Simm's ultrafiltrate of beef serum,[5] citrated human plasma from the hospital blood bank and 9 to 11 day old chick embryo extract in isotonic solution of sodium chloride. The human plasma is allowed to stand in the cold until a cloudy precipitate forms, which is discarded.

The flasks are put in an incubator at 37 C. They are turned once every twenty-four hours. The nutrient is replaced every two to three days.

RESULTS

The clot formed by this method remains clear for at least three weeks. Later fine fibrils may be seen microscopically. The clot does not prevent accurate microscopic observation. The fibrillar network does not hinder the growth of cells. It forms an excellent framework for the preservation of very long processes such as those seen in nerve elements. On the other hand, it can be easily removed so that the cells adherent to the glass are undisturbed and can be preserved and stained.

There is no evidence that the fibrinogen, the thrombin or the ingredients therein are toxic to the cells.[6] Luxuriant growth of glial elements from normal human brain and of cells from normal human testis has been observed within eight hours, and the glass cover slips were covered with cells within forty-eight hours, when the fibrinogen and the thrombin were used in high concentrations. At times the powdered thrombin has been placed directly on the cells without apparent injury, as evidenced by continued proliferation of fibrocytes.

SUMMARY

A method is described for the use of purified fibrinogen and thrombin to form a clot in tissue culture preparations.

5. Simms, H. S., and Sanders, M.: Arch. Path. **33**:619, 1943.
6. Ingraham, F. D., and Bailey, O. T.: J. A. M. A. **126**:680, 1944.

Notes and News

Fourth International Cancer Research Congress.—This congress was held in St. Louis from September 2 to 7, under the joint auspices of the Union Internationale Contre le Cancer and the American Association for Cancer Research. Dr. E. V. Cowdry was president of the congress. Official delegates were present from forty-four countries. Also in attendance were many enthusiastic foreign and domestic guests. Eight general sessions, each addressed by three speakers, and about thirty special sessions, with numerous shorter papers being presented, were held. Sessions were devoted to pathology and diagnosis, chemotherapy, isotopes, radiation biology and therapy, carcinogenic hydrocarbons, cancer genetics, the biology, cytology, chemistry and etiology of cancer, hormones, milk factor, comparative oncology and other subjects. The announcement delivered by telegram to the congress from President Truman that radioactive isotopes had been released for research to qualified foreign research workers was greeted with enthusiasm. Plans were drawn for a permanent world organization to combat cancer. The broad scientific attack on cancer problems which is now under way was apparent, and the announced results were encouraging and stimulating. It is expected that the papers will be published shortly.

Eye Foundation.—At St. Vincent's Hospital, in Los Angeles, Mrs. E. L. Doheny has established the Estelle Doheny Eye Foundation to provide comprehensive ophthalmic laboratory and research facilities. A. Ray Irvine, professor of ophthalmology at the University of Southern California, is chairman of the advisory committee. P. Soudakoff, formerly associate professor of ophthalmology in Peking Union Medical College, is the pathologist on full time.

Cancer Professorship.—Cornell University Medical College has established a professorship of cancer, which has been endowed with $15,000 a year for five years. This represents the first allocation of funds collected in the 1947 campaign of the New York City Committee of the American Cancer Society.

Society News.—The American College of Physicians will conduct its twenty-ninth annual session at San Francisco, April 19 to 23, 1948. General headquarters will be at the Civic Auditorium. Secretaries of medical societies are especially asked to note these dates and, in arranging meeting dates of their societies, to avoid conflicts with the college meeting, for obvious mutual benefits.

The American Association for the Advancement of Science will hold its one hundred and fourteenth meeting, December 26 to 31, in Chicago.

The American Association for the Study of Goiter will meet in the King Edward Hotel, Toronto, Canada, May 6, 7 and 8, 1948.

Research Fellowships.—The American College of Physicians announces that a limited number of fellowships in medicine will be available from July 1, 1948 to June 30, 1949. These fellowships are designed to provide an opportunity for research training either in the basic medical sciences or in the application of these sciences to clinical investigation. They are for the benefit of physicians who are in the early stages of their preparation for a teaching and investigative career in internal medicine. The stipened will be from $2,200 to $3,000. Application forms may be obtained from the American College of Physicians, 4200 Pine St., Philadelphia 4.

Books Received

CHEMICAL CARCINOGENESIS. By Alexander Haddow and others. Price 10 s. London, W.1: Medical Department, The British Council, 1947.

Two numbers (5 and 6 under one cover) of the *British Medical Bulletin* (4:309-444, 1947), a comparatively new publication, are devoted to eighteen reviews under the general title "Chemical Carcinogenesis." Twenty different authors present some of the results of the research supported in part by the British Empire Cancer Campaign over more than twenty years in eleven centers.

Haddow and Kon review the history and the salient features of the chemistry of carcinogenic compounds. Physical methods which have proved highly useful in investigations of carcinogenic hydrocarbons are briefly discussed by Berenblum, Holiday and Jope, who give the limitations of each technic. In a section on the mechanism of carcinogenesis, Haddow critically reviews the modes of action of these chemicals, while Berenblum and Crabtree discuss cocarcinogenesis and anti-carcinogenesis and Dickens delves into the disturbing problem of the influence of the solvent on the carcinogenic response. The metabolism of carcinogenic compounds is reviewed by Boyland and Weigert. In a section on carcinogens of biologic origin, those originating from human tissues are reviewed by Hieger, and the carcinogenic action of heated fats and lipids is critically discussed by Peacock. Under the subtitle of remote carcinogenic action, estrogens are reviewed by Burrows and Horning, stilbene derivatives by Dodds, 2-acetylaminofluorene and related compounds by Bielschowsky, azo compounds by Orr and experimental cancer of the bladder by Bonser. Occupational cancers also receive attention: those of the skin, from Henry; those of the bladder, from Goldblatt, and those due to arsenic, from Currie. In the final section Haddow deals with chemotherapy.

Enumeration of the papers reveals the wide range of subjects covered, but it does not do justice to their high interest or quality. Throughout the papers runs a note of confidence that work with chemical carcinogens is not finished but only beginning and that, however productive the past, the future should be brighter. They leave the impression that these men know cancer, understand the enemy and are making their attack wisely.

RECENT ADVANCES IN ENDOCRINOLOGY. By A. T. Cameron, C.M.G., M.A., D.Sc. (Edin.), professor of biochemistry, University of Manitoba Faculty of Medicine; biochemist, Winnipeg General Hospital. Sixth edition. Pp. 443, with 74 figures, including 3 plates. Price $6. Philadelphia and Toronto: The Blakiston Company, 1947.

The previous edition of this compact book was published two years ago. Since that time "no outstanding advance has been made," but there has been steady progress in endocrinology, requiring revisions and additions. The book continues to merit its popularity.